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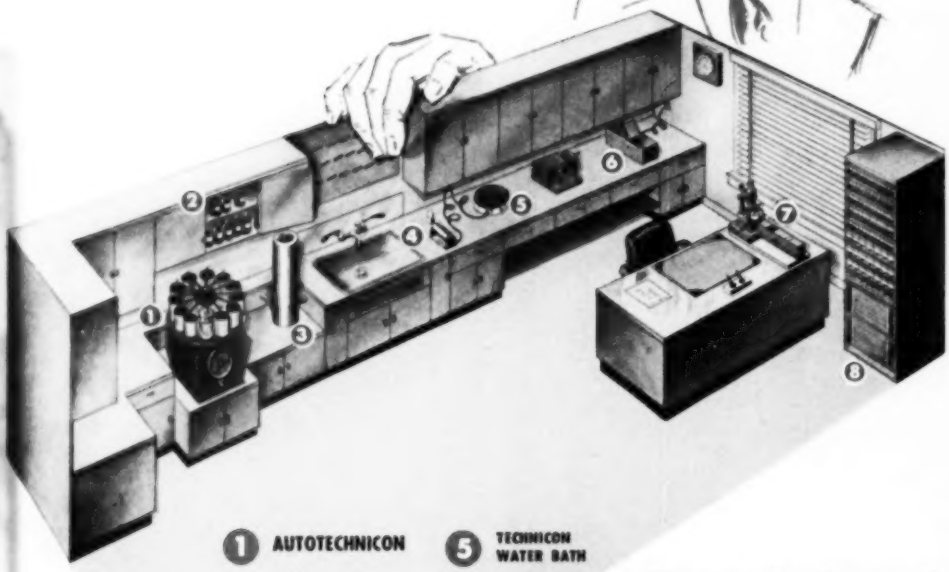

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ARTERIOSCLEROSIS IN THE DOG

I. Spontaneous Lesions in the Aorta and the Coronary Arteries

STUART LINDSAY, M.D.
SAN FRANCISCO
I. L. CHAIKOFF, M.D.
BERKELEY, CALIF.
AND
J. W. GILMORE, D.V.M.
SAN MATEO, CALIF.

A NUMBER of papers have dealt with arteriosclerosis of the dog. One of the earliest reports was that of Köllisch,[1] who found degenerative disease in the aortas of 23 of 100 dogs. Strauch [2] examined 56 dogs, 9 of which were 5 to 8 years of age, and concluded that while arteriosclerosis was found rarely in young dogs, most older animals were afflicted, especially after 8 years of age. He believed that arteriosclerosis resulted from infection, after a prolonged incubation period. The arterial lesions appeared grossly as streaks or oval plaques, and were most numerous in the abdominal aorta near the bifurcation or near vascular ostia. He found, in addition, fragmentation and consistent splitting of the internal elastic membrane; the intimal thickening noted was a proliferative process, followed by hyalinization without necrosis. Although he observed true atherosclerosis in the femoral arteries of older dogs, he found that fatty degenerative lesions of the aorta seldom occurred. He attributed this to the fact that plasma constituents of the dog differ from those of man.

Krause [3] noted the frequency of abdominal aortic plaques in dogs after the fifth year and reported that the lesions were the result of hyperplasia of the intimal connective tissue associated with the appearance of fine fibrils, some of which were elastic in nature.

In a comparative study of arteriosclerosis, Nieberle [4] found only small and large calcifications in the arterial media of dogs.

Zinserling,[5] from a study of 26 dogs ranging in age from 8 to 28 years, concluded that when lipid is deposited in the arterial wall, its deposition is preceded

This study was aided by a grant from the United States Public Health Service.

From the Divisions of Pathology (Dr. Lindsay) and Physiology (Dr. Chaikoff) of the University of California School of Medicine.

1. Köllisch, P.: Zur pathologischen Anatomie und Aetiologie der sogenannten Atherosklerose der Arterien bei den Haustieren, Inaugural dissertation [Bern], Nürnberg, J. L. Stich, 1910.

2. Strauch, C.: Beitr. path. Anat. **61**:532, 1916.

3. Krause, C.: Beitr. path. Anat. **71**:121, 1922.

4. Nieberle, K.: Verhandl. deutsch. path. Gesellsch. **25**:291, 1930.

5. Zinserling, W. D.: Beitr. path. Anat. **88**:241, 1932.

by intimal sclerosis. Although the locations of the lesions were the same in man and dog, Zinserling believed that the human lesion differed from that of the dog because in the former the lipid infiltration was a primary phenomenon. Alterations in the canine media were found rarely and were not necessarily associated with intimal lesions. Zinserling further concluded that alteration of the connective tissue of the vascular wall, as well as cholesterol deposition, was important in the pathogenesis of canine arteriosclerosis.

A more recent study of arteriosclerosis in otherwise normal dogs is that of Morehead and Little.[6] A variety of lesions of intima and media were described. Most dogs, even 10-day-old puppies, had localized intimal plaques associated with splitting and reduplication of the internal elastic lamellae. Medial disease was more pronounced and was limited to the aortas of older dogs. It consisted of focal loss of elastic tissue with grouping and hyperplasia of smooth muscle cells, medial necrosis with cyst formation, and localized fibrosis with calcification. Although hyaline deposits were observed in the intima of renal arteries, minimal intimal fibrosis was seen in only one large coronary artery.

The present report is based on examinations of aortas from 26 dogs, 14 of which were 8 to 17 years old. In addition to aortic disease, microscopic evidence of coronary arteriosclerosis, at times associated with thrombosis or myocardial infarction and scarring, was found in 10 of the 14 aged dogs. Myocardial failure, resembling closely that occurring in man, was observed in five of the dogs. An important finding brought out by this study is that *lipid material is not associated with the earliest aortic disease in the dog,* and this point is discussed in relation to the etiology of canine arteriosclerosis.

MATERIALS AND METHODS

Twenty-six dogs were used in this study (table). Twelve of the dogs (Group 1) were mongrels of unknown age. They were surplus animals in the laboratory and had not been subjected to any experimental procedures. Their sexes were not recorded at the time of autopsy. Fourteen of the dogs (Group 2) were obtained from a veterinary hospital. These dogs varied in age from 8 to 17 years, the majority being 12 and 13 years old. There were 11 males and 3 females. Twelve were purebred dogs, equally divided between large and small breeds. The two mongrel dogs in the group were large animals. Five of the dogs were ill with chronic cardiac failure, and seven were killed because of advanced age. One had a postencephalitic syndrome, and another had a large osteogenic sarcoma. The dogs in both groups were killed by intravenous injection of pentobarbital sodium U. S. P.

In Group 1 the aorta was the only tissue removed and examined; the abdominal aorta was examined in only 4 of the 12 dogs. In Group 2 the heart, the entire aorta, and the common iliac arteries were removed for study. The vascular apparatus was opened longitudinally, and its gross appearance was noted. After fixation in 10% formaldehyde solution U. S. P., blocks of tissue were removed from each aorta at the following levels: apex of arch, midthoracic region, and lower abdominal region approximately 2 to 3 cm. above the aortic bifurcation. In a few instances, blocks of tissue were removed at other levels, particularly if lesions were grossly visible. Contiguous frozen sections from each block were stained with (1) Sudan IV and hematoxylin and (2) Nile blue. An unstained frozen section was used for examination with polarized light. These blocks were then embedded in paraffin, and contiguous sections were treated with hematoxylin and eosin stain, Laidlaw's connective tissue stain, and a combined Weigert-Van Gieson stain. Selected sections were also treated with a modified iron-Prussian stain for mucopolysaccharides.[7] Blocks of tissue were removed from the hearts of the dogs of the second group;

6. Morehead, R. P., and Little, J. M.: Am. J. Path. **21**:339, 1945.

7. Rinehart, J. F., and Abul-Haj, S. K.: An Improved Method for Histologic Demonstration of Acid Mucopolysaccharides in Tissues, A. M. A. Arch. Path. **52**:189 (Aug.) 1951.

Group	Breed	Sex and Age, Yr.	Clinical Diagnosis	Thoracic Aorta: Gross Appearance: Plaques	Thoracic Aorta: Gross Appearance: Size, Mm.	Thoracic Aorta: Gross Appearance: Color	Thoracic Aorta: Microscopic Examination: Intimal Thickening†	Thoracic Aorta: Microscopic Examination: Medial Disease†	Thoracic Aorta: Microscopic Examination: Lipids‡	Thoracic Aorta: Microscopic Examination: Cholesterol§	Abdominal Aorta: Gross Appearance: Plaques	Abdominal Aorta: Gross Appearance: Size, Mm.	Abdominal Aorta: Gross Appearance: Color	Abdominal Aorta: Microscopic Examination: Intimal Thickening	Abdominal Aorta: Microscopic Examination: Medial Disease	Abdominal Aorta: Microscopic Examination: Lipids‡	Abdominal Aorta: Microscopic Examination: Cholesterol§
1	Mongrel	Unknown	Normal	0	...	.....	0	++	0	0	Several	1	White	++	0	0	0
	Mongrel	Unknown	Normal	0*	...	.....	0	++	0	0	#	...	.....	....	..	..	..
	Mongrel	Unknown	Normal	Several	1	White	+	+++	0	0	#	...	.....	....	..	..	..
	Mongrel	Unknown	Normal	0*	...	.....	+	+++	+	0	#	...	.....	....	..	..	..
	Mongrel	Unknown	Normal	Few	1	White	0	+++	0	0	#	...	.....	....	..	..	..
	Mongrel	Unknown	Normal	0	...	.....	+	++++	0	0	2	0.5	White	++	++	0	0
	Mongrel	Unknown	Normal	0	...	.....	+	++++	0	0	Several	0.5-1	White	+	0	0	0
	Mongrel	Unknown	Normal	1	5	White	++	+++	0	0	#	...	.....	....	..	..	..
	Mongrel	Unknown	Normal	1	5	White	++	++	0	0	0	...	.....	+	0	0	0
	Mongrel	Unknown	Normal	Few	5	White	0	+++	0	0	#	...	.....	....	..	..	..
	Mongrel	Unknown	Normal	0	...	.....	0	+++	0	0	#	...	.....	....	..	..	..
	Mongrel	Unknown	Normal	0	...	.....	0	+++	0	0	#	...	.....	....	..	..	..
2	Cocker spaniel	M, 13	Postencephalitic syndrome	Numerous	1-2	White	+++	+++	0	0	0	...	.....	+++	0	0	0
	Setter	M, 17	Chronic cardiac failure	Numerous	15-20	White	++	+++	+	0	1	5	White with hemorrhage	+++	++	0	0
	Cocker spaniel	M, 8	Osteogenic sarcoma	Numerous	1-2	White	+	+++	0	0	Numerous	1-2	White	+++	++	0	0
	Wire-haired terrier	M, 12	Chronic cardiac failure	Numerous	1-3	White	+	+++	0	0	Numerous	1-3	White	+++	+	+	0
	Welch terrier	M, 12	Chronic cardiac failure	0	0	0	0	++	0	0	Several	0.5-1	White	+	0	0	0
	German shepherd	M, 13	Advanced age	Few	1-5	White	+	++	+	0	Few	1-5	White	++	0	0	0
	Mongrel	M, 12	Advanced age	Numerous	7-20	White	+++	+	+	0	Few	1.5-1	White	+++	0	+	0
	Mongrel	M, 11	Advanced age	1 in sinus of Valsalva	1-6	Pale yellow	++	++	+	0	Several	1-2	White, few yellow	+++	0	+++	0
				Few	10-20	White	0	+	0	0	.....	...	.....	....	..	..	..
3	Afgan hound	M.	Chronic cardiac failure	3	5-1	White	+	0	0	0	Numerous	0.5-1	White	+++	+	++	0
	Shepherd	F, 17	Chronic cardiac failure	Numerous	5	White	++	0	+	0	Numerous	5-10	White	+	0	++	0
	Shepherd	M, 12	Advanced age	Diffuse thickening	...	White	+++	+	+++	0	Diffuse thickening	...	White	+++	0	++	0
				Several in sinus of Valsalva	1	Pale yellow	..	..	..	..	.....	...	.....	....	..	..	..
	Fox terrier	F, 12	Chronic cardiac failure	0	0	0	0	0	0	0	1	5	White	++	0	0	0
	Scotch terrier	M, 13	Advanced age	Numerous	Coalescing	White	++	0	0	0	Several	1-2	White	++	0	0	0
	Shepherd	F, 12	Advanced age	Numerous	1-2	White	+	0	+	0	Numerous	1-2	.....	+++	0	++	0

* Slight intimal wrinkling without plaque formation was observed.
† One plus means minimal amounts in intima; 2+, moderate amounts in intima; 3+, abundance in intima as well as in adjacent media.
‡ As judged by Sudan IV and Nile blue stains.
§ As judged by polarized light and Nile blue stain.
# This sign means the animal was not examined in regard to the detail in question.

multiple sections were taken from both ventricular walls and the major coronary arteries. After being embedded in paraffin, these blocks were sectioned and stained with hematoxylin and eosin. A few sections were stained for mucopolysaccharides.

GROSS DESCRIPTION

GROUP 1. DOGS OF UNKNOWN AGES

In general, the aortas from these dogs had fewer and smaller intimal lesions than did those of the older dogs in Group 2.

Thoracic Aorta.—Seven of the 12 dogs of Group 1 had distinct lesions of the thoracic aorta. In two dogs the alteration was limited to a slight thickening and longitudinal wrinkling of the intima of the arch. In two other animals there were a few small white linear or streak-like plaques lying beneath the openings of the intercostal arteries. In one dog the plaques adjacent to vascular openings tended to be triangular. Two other dogs had single, irregular areas of puckering and intimal scarring localized to the convex surface of the aortic arch. In each instance, these scarred zones measured 0.5 cm. at their widest diameters. The thoracic aortic lesions were white and showed no gross evidences of lipid infiltration.

Abdominal Aorta.—Abdominal aortas were removed from only four dogs; three of the aortas had sclerotic lesions. In each of these three there were small plaques measuring between 0.5 and 1 mm. in diameter; they were white, few in number, round or elongated, and often lay adjacent to arterial openings. None showed evidences of intimal lipid infiltration.

GROUP 2. DOGS OF KNOWN AGE

Heart.—Grossly demonstrable cardiac lesions were found in two of the older dogs. Both were 12 years of age, and both had been ill with chronic cardiac failure. In one dog there was a depressed area (1 cm. in diameter) of fibrosis involving the endocardium and the adjacent myocardium of the posterior portion of the left ventricular wall. In the other dog there was an extensive area of infarction involving the apex of the left ventricle; this damaged area measured 4 cm. in diameter and presented alternating zones of opaque necrosis, translucent fibrosis, and congestion.

Thoracic Aorta.—Arteriosclerotic lesions were found in all segments of the thoracic aorta in all but two animals. These lesions were usually slightly elevated, multiple, and appeared white and fibrous. They varied from 1 to 2 mm. up to 2 cm. in diameter. Their outlines were generally distinct, but some were poorly demarcated from the surrounding intima. They tended to be oval and elongated, although in a few instances they had circular outlines. They were most numerous in the arch and larger on the concave surface. In three dogs these plaques had distinctly narrowed the coronary orifices—the right orifice in two dogs and the left orifice in the third. In the descending portion of the thoracic aorta, the plaques were most pronounced about the intercostal openings, which often were distinctly narrowed. In the aorta of one dog there was a diffuse thickening of the intima, almost localized to the segment between the intercostal orifices. In the aortas of two dogs a few tiny, punctate, elevated, yellow plaques were seen. In one dog a sinus of Valsalva contained a single, pale, fibrous plaque, which measured 1 by 6 mm. Another dog had a single, pale yellow plaque, 1 mm. in diameter, in one of the sinuses of Valsalva.

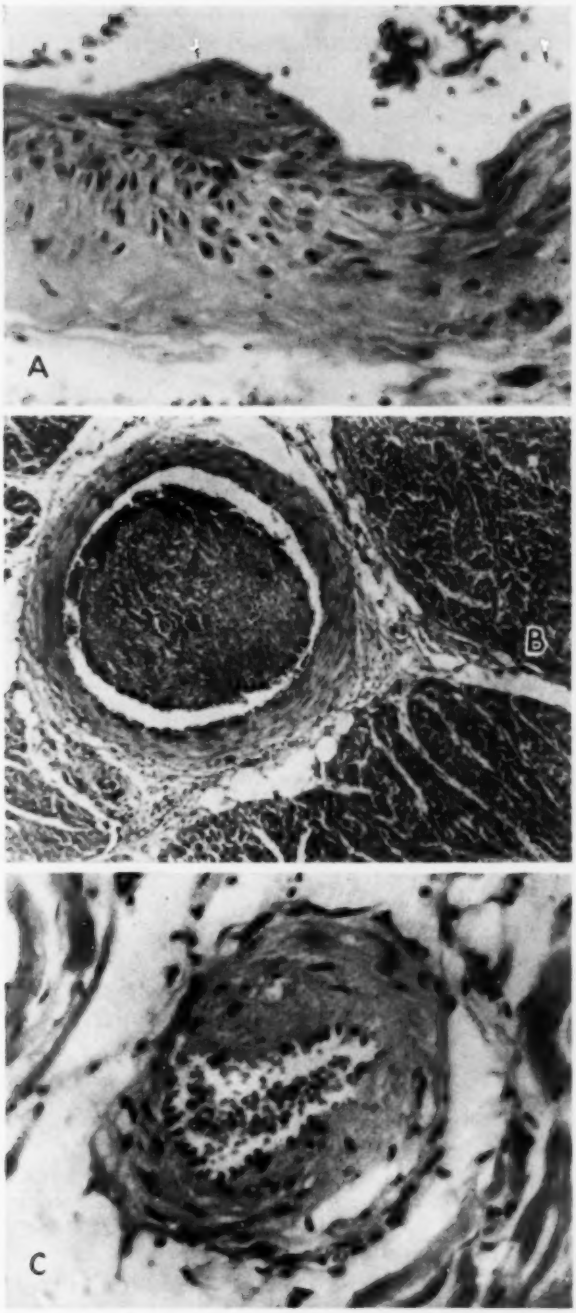

Fig. 1.—*A*, intimal plaque of a main coronary artery. Hematoxylin and eosin; × 320.

B, coronary thrombosis with infarction of the adjacent myocardium. Hematoxylin and eosin; × 80.

C, intimal fibrous thickening of small coronary artery with hyalinization. Hematoxylin and eosin, × 320.

Abdominal Aorta.—All but one dog had arteriosclerotic lesions of the abdominal aorta. These resembled, as a rule, the ones seen in the thoracic portion. They were oval, round, or, at times, elongated, and varied from 1 mm. to 1 cm. in diameter. At times they were confluent. They were generally more numerous and larger on the posterior wall and became progressively more numerous and

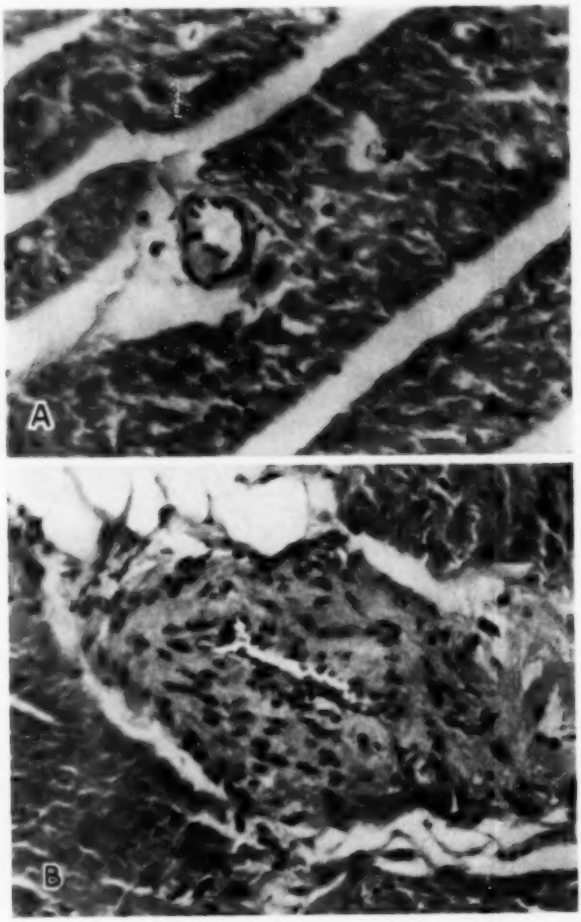

Fig. 2.—*A*, eccentric hyaline plaque in small coronary artery. Hematoxylin and eosin; × 320. *B*, coronary artery with muscular hypertrophy. Hematoxylin and eosin; × 320.

abundant toward the lower portions of the aortic segment. In several dogs the confluence of linear, streak-like plaques had resulted in a trabeculated appearance of the intima, with the striations tending to fall in a transverse manner. When the plaques were fewer and smaller, they tended to be congregated about the

openings of arteries. In one dog a single, round, slightly elevated plaque, measuring 0.5 cm. in diameter, contained a zone of antemortem hemorrhage.

MICROSCOPIC DESCRIPTION

Coronary Arteries

In 10 of the 14 dogs of Group 2 there was microscopic evidence of disease of the heart involving the myocardium, the valves, and the coronary arterial system. In three of the dogs distinct lesions of the major coronary arteries were found. In two there were small elevated fibrous intimal plaques (Fig. 1*A*). The plaques from one dog contained a few lymphocytes; those from the other were composed of loosely arranged cells that appeared to be enclosing small vascular spaces. Two large coronary arteries were occluded by organizing thrombi associated with hemorrhage, fibrinoid degeneration, and chronic inflammation of the vascular walls. These lesions were seen adjacent to a zone of myocardial infarction in one of the animals (Fig. 1*B*). In three dogs changes were found in the medium-sized coronary arteries; in two this consisted of intimal fibrous thickening, usually with considerable hyaline degeneration (Fig. 1*C*). In some vessels there was plaque formation with an eccentric arrangement. In others the intimal thickening was arranged concentrically. Alteration of small coronary arteries was observed in four dogs. In all instances this consisted of eccentrically placed hyaline intimal plaques which projected into, and partially occluded, the vascular lumens (Fig. 2*A*). An occasional small artery showed muscular hypertrophy, at times associated with the presence of Anitschkow myocytes in the adventitial layer (Fig. 2*B*). In a small intimal plaque in one major coronary artery, a small calcific deposit was present. An occasional medium-sized artery showed considerable vacuolation of the cytoplasm of the cells comprising the media.

In one dog, several large myocardial arteries were extensively damaged (Figs. 3*A* and *B*). Their intimal layers showed pronounced hyaline fibrous thickening, which encroached on the media. Within these plaques some of the connective tissue cells adjacent to the endothelium resembled Anitschkow myocytes. The lumens were considerably narrowed. Macrophages containing pigment were often noted in the adventitia of these arteries. This hyaline material in the intima had entirely replaced the internal elastic membrane except for a few small segments. In general, it was homogeneous. Some portions appeared more fibrillary, and these stained metachromatically with toluidine blue. The hyaline substance stained distinctly with the iron-Prussian blue method for mucopolysaccharides.[7] None was removed by incubation with testicular hyaluronidase. This vascular abnormality in this one dog was limited to the left ventricular wall and was associated with focal myocardial fibrosis and lymphocytic infiltration.

Myocardium

Five of the dogs of Group 2 had lesions of the myocardial layer limited to the left ventricular wall. In one animal there was a triangular myocardial scar lying adjacent to the endocardium of the left ventricle (Fig. 4). This contained numerous small capillary channels and consisted of delicate fibrous tissue. Many macrophages filled with pigment and lymphocytes were present. A second dog had small foci of degeneration of myocardial fibers associated with lymphocytic infiltration. In another animal the myocardial abnormality was limited to a few smaller areas

where fibrous replacement of muscle had occurred. In the fourth dog the only abnormalities noted were several small foci of perivascular lymphocytic infiltration.

The fifth dog showed extensive areas of myocardial infarction (Fig. 1*B*) which appeared as large zones of necrosis of myocardial fibers associated with a dense infiltration of neutrophilic leucocytes. The surrounding myocardium was exten-

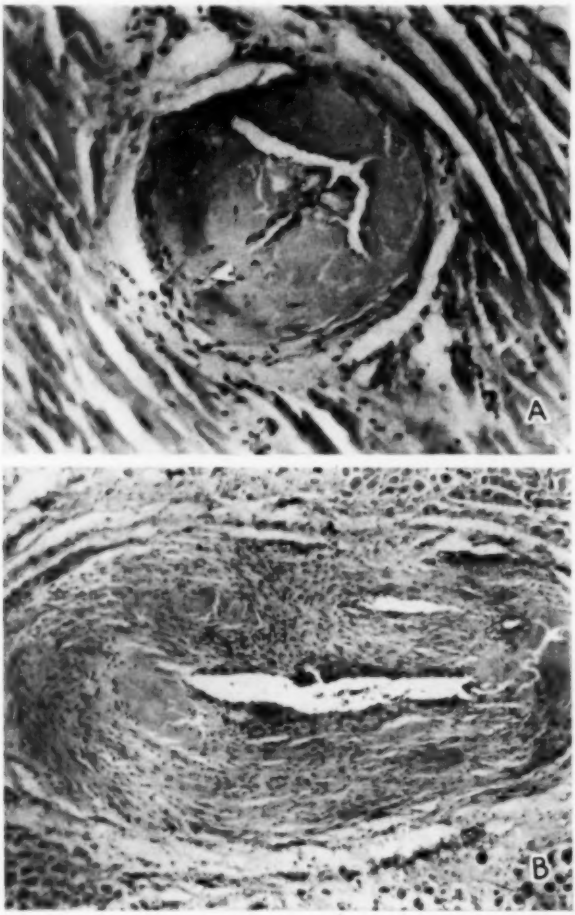

Fig. 3.—*A*, coronary artery with extensive hyaline deposits. Hematoxylin and eosin; × 160. *B*, coronary arteries with focal hyaline deposits. Hematoxylin and eosin; × 80.

sively scarred and was infiltrated with acute and chronic inflammatory cells. Hemorrhage had occurred in the necrotic portions of the lesion. The more extensive myocardial lesions were found in dogs in which the coronary vascular disease was more pronounced.

Valves

A valvular lesion was seen in only one of the dogs of Group 2. At the base of the mitral valve there were collections of lymphocytes and large mononuclear cells. The distal portion of the valve consisted of loosely arranged, delicate fibrous tissue. The myocardium adjacent to the valvular attachment showed an area of scarring with calcific deposits (Fig. 4*B*).

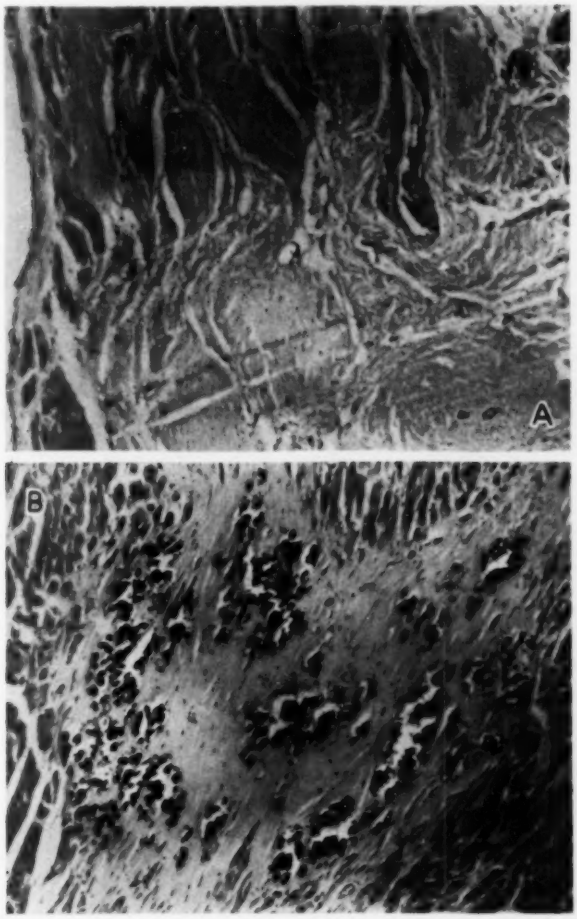

Fig. 4.—*A*, myocardial scar. Hematoxylin and eosin; × 80.
B, myocardial fibrosis and calcification. Hematoxylin and eosin; × 80.

Aorta

General Characteristics.—The 12 dogs in the younger age group (Group 1) all had microscopic lesions of the aorta, either of the intima or of the media or of both. The 14 older dogs (Group 2) all had microscopic evidences of intimal

disease, and the majority showed abnormalities of the media as well. Two of the older dogs revealed neither gross nor microscopic lesions of the intima of the thoracic aorta; one dog showed no gross lesions of the abdominal aorta, but extensive disease in this region was found by microscopic study.

Intima—(*a*) General Appearance: The arterial lesions seen in the two groups of dogs were similar. Although the structure of the media is different in the thoracic and abdominal segments in the dog, the lesions in each area were identical. Medial elastic fibers are fewer in the abdominal aorta, and the muscular elements are more abundant.

The earliest evidence of disease of the intima was an alteration of the internal elastic membrane. This consisted of fraying or splitting, often associated with distinct reduplication. In many sections, fragmentation or even loss of the internal elastic membrane was observed even in the absence of other demonstrable intimal alteration (Fig. 5).

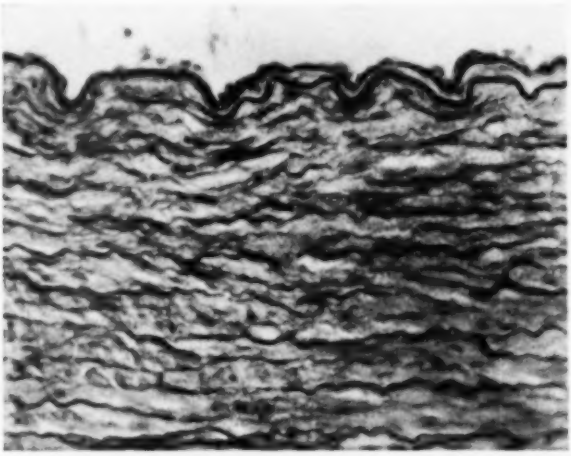

Fig. 5.—Abdominal aorta showing reduplication of internal elastic lamina without intimal thickening. Weigert-Van Gieson stain; × 320.

The characteristic intimal lesion consisted of a fibrous thickening which in its more pronounced forms led to formation of intimal plaques. In vessels showing multiple, large plaques, the intima between them was generally also slightly or moderately thickened. The fibrous tissue comprising the plaques varied in appearance. The smaller plaques or early lesions of intimal thickening were usually composed of loosely arranged fibroblastic cells and fibers in haphazard arrangement (Fig. 6*A* and *B*). Often the long axis of the cells and their fibers lay at right angles to the circumference of the vessel. More frequently, particularly in the larger plaques, the cells and fibers were oriented in a circumferential manner (Fig. 6*C*). The looseness of the cellular and fibrillar arrangement was more pronounced in frozen than in paraffin sections. In a few early intimal plaques the fibroblastic cells were obviously immature; they possessed hyperchromatic nuclei (Fig. 6*B*) and frequently showed distinct mitotic figures. In the larger plaques,

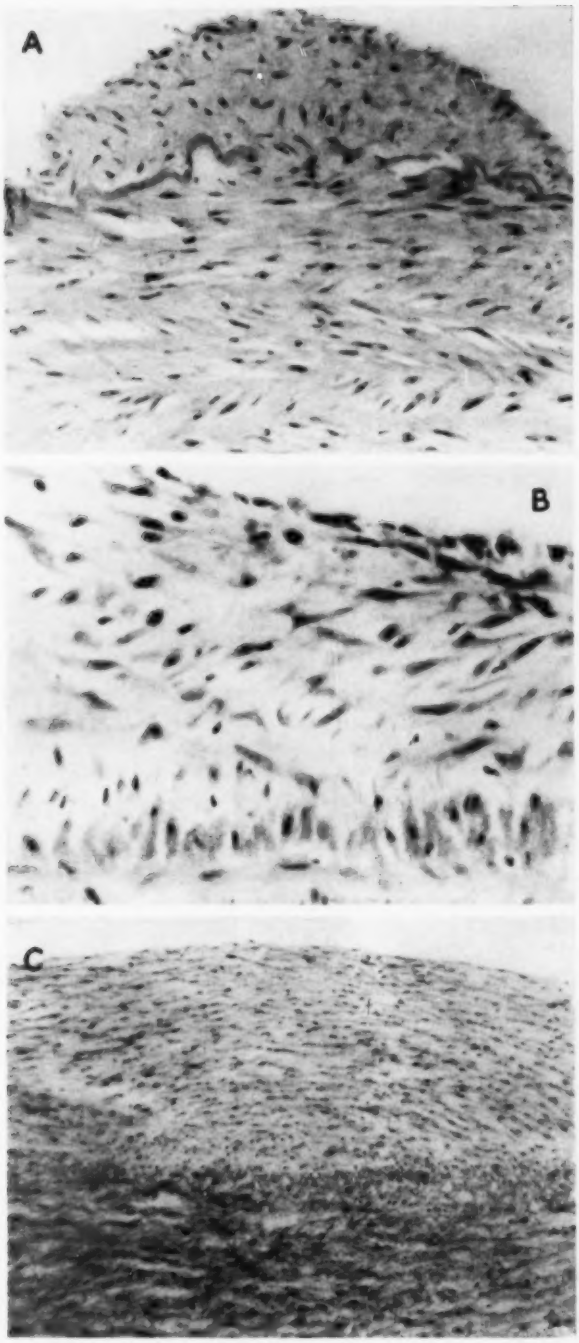

Fig. 6.—*A*, abdominal aorta showing early intimal plaque with irregular cellular arrangement. Hematoxylin and eosin; × 160.

B, abdominal aorta showing intimal fibrosis with irregular cellular arrangement. Hematoxylin and eosin; × 320.

C, thoracic aorta showing intimal thickening with circumferential cellular arrangement. Hematoxylin and eosin; × 80.

having transversely arranged cells and fibers, the fibrillary structures had a wavy character, probably due to postmortem medial contraction.

The larger plaques usually had a laminated structure (Figs. 7*A* and 8*A*). The deeper portions presumably were older and consisted of more compact, coarser fibrils and inactive fibrocytes. The inner portions of these plaques, on the other hand, had a looser arrangement, and the cells were less mature. The fibrils were fewer and also less mature. Zones of acellularity accompanied with hyalinization were occasionally seen. In some of the larger plaques, three or four distinct layers were visible. Each layer was progressively more compact from the lumen outward. In those vessels in which the entire circumference was involved by intimal disease, the cells and fibers of the thickened intima often had both a perpendicular and a circumferential arrangement.

In three instances, deeper portions of intimal plaques had a distinct mucoid stroma. In other plaques there was wide separation of the cells and fibers that gave the tissue an edematous appearance. Usually the degree of intimal thickening was greater near the openings of arterial branches of the aorta. In one large intimal plaque there was a zone of organizing hemorrhage in the deeper portion. In another large intimal plaque there were irregular deposits of eosinophilic hyaline material, and this material stained only faintly with Sudan IV and Nile blue. In one aorta there were small calcific deposits in the thickened intima; these were punctate and were congregated about the intimal fibroblasts (Fig. 7*C*).

In an early plaque in one of the younger dogs (Group 1) the intimal fibroblasts had unusually distinct cellular outlines, and their cytoplasm was vacuolated. Only one cell, however, contained demonstrable lipid material.

(*b*) Elastic and Connective Tissues: The Weigert-Van Gieson stain revealed considerable alteration of the elastic tissue of the intimal layer. Even in the absence of intimal thickening the internal elastic membrane occasionally showed fraying, splitting, or fragmentation. This membrane was occasionally absent. These changes were visible beneath the zones of intimal thickening and beneath intimal plaques (Fig. 8*A*). In one instance a frayed internal elastic membrane plus a few transversely arranged elastic fibers had formed a very early intimal plaque-like lesion. In other instances the altered internal membrane which was reduplicated formed part of larger intimal plaques. At the margin of one of the intercostal arterial openings, fragmentation and beading of the internal elastic membrane were present without associated intimal fibrosis or thickening.

In the most recently formed, inner portions of intimal plaques, elastic fibers were generally absent. They were present, however, in the deeper, older portions of the plaques (Fig. 8*A*). In the deepest segments, adjacent to the media, they were most abundant, tended to be laminated or wavy, and were often as coarse as the underlying adjacent internal elastic membrane. The finer elastic tissue fibrils in the earlier plaques tended to surround the fibroblastic cells and were intimately associated with their cytoplasm (Fig. 8*B*). In a few instances, elastic fibers were coarser and more numerous in the superficial portions of the plaque than in the deeper segments. In some plaques the thicker, coarser elastic fibers were degenerating and had a fragmented or granular appearance.

The Laidlaw connective tissue stain revealed delicate reticulum fibers or coarse, wavy, collagenous fibers in the intimal plaques. The more delicate fibers were closely associated with the cytoplasm of the fibroblastic cells, and the direction of

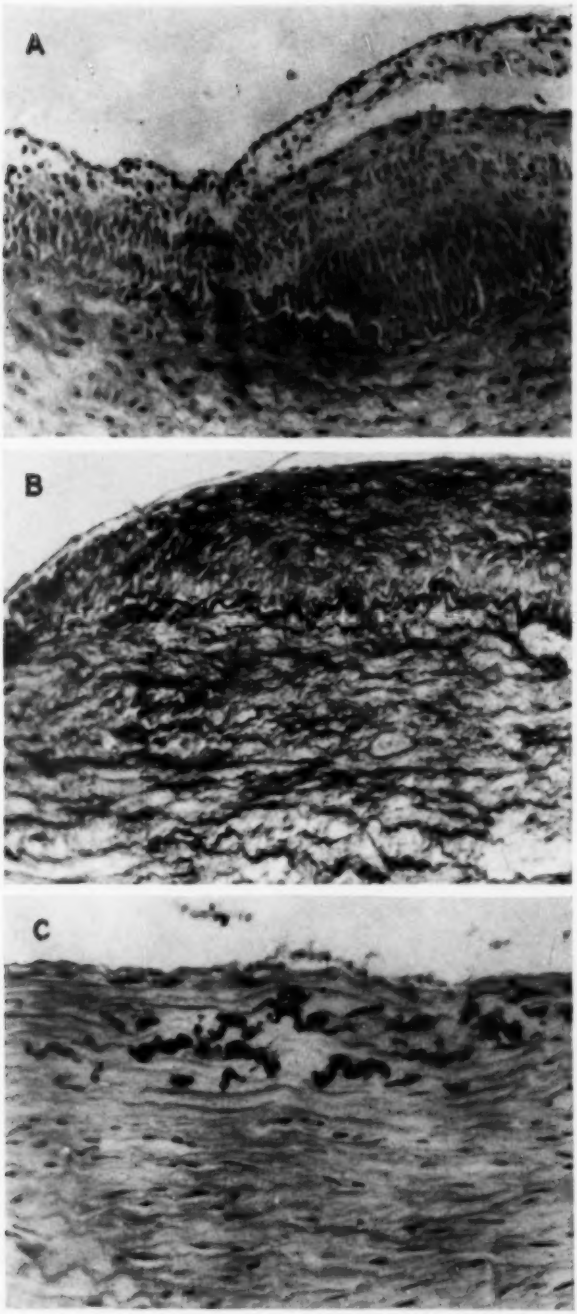

Fig. 7.—*A*, abdominal aorta showing layering of intimal plaque. Hematoxylin and eosin; × 160.

B, thoracic aorta showing alteration of internal elastic membrane beneath intimal plaque. Weigert-Van Gieson stain; × 160.

C, abdominal aorta showing calcific deposits in intima. Hematoxylin and eosin; × 160.

the fibers of either type was the same as that of the long axis of the fibroblasts or fibrocytes.

(*c*) Lipid Material: In Group 2 a few sudanophilic droplets in a single, swollen intimal connective tissue cell in one dog were the only evidence of lipid infiltration. However, in the older dogs (Group 2) minimal amounts of lipid

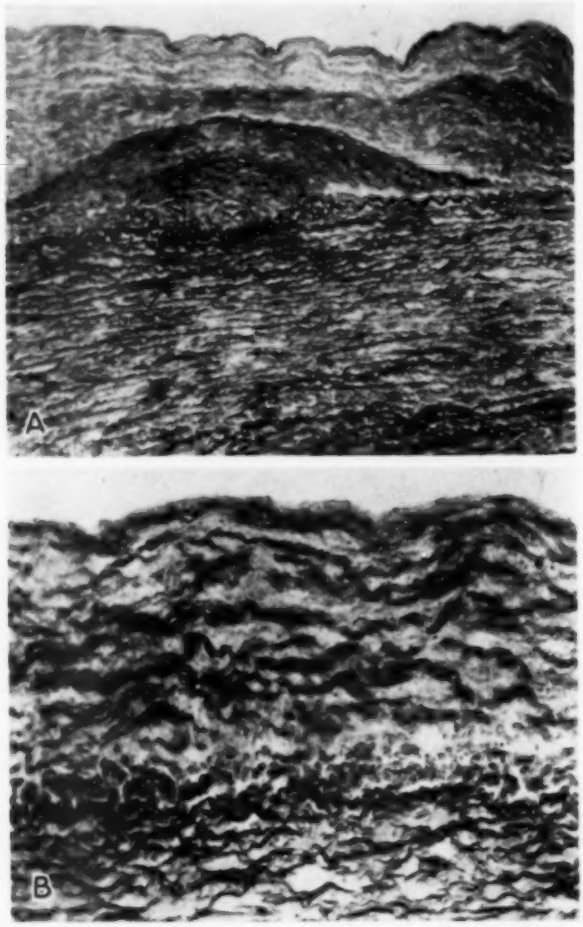

Fig. 8.—*A*, abdominal aorta showing lamination of intimal plaque and development of intimal elastic fibers. Weigert-Van Gieson stain; × 160.

B, thoracic aorta showing delicate pericellular elastic fibers in intima. Weigert-Van Gieson stain; × 160.

material were found in 9 of the 14. This appeared as small orange droplets in the cytoplasm of the fibroblastic cells of the thickened intima. This finely divided lipid could not be photographed satisfactorily. In most instances the lipid material was most abundant in the deeper portions of the plaques. In those vessels in which

only minimal amounts were demonstrable, it tended to be deposited along the internal elastic membrane. In a few instances the lipid was most abundant superficially near the endothelium. When most abundant in the thickened intima or in the intimal plaques, droplets of lipid were also present in the connective tissue cells of the immediately adjacent media. This material stained less distinctly with Nile blue and varied from pale violet to deep blue. In several dogs the amount of lipid material was considerable.

In none of the dogs was refractile material demonstrable either in the intimal or in the medial layers when these were examined with polarized light.

(*d*) Mucopolysaccharides: Certain selected sections of the aorta were studied after they had been stained by the iron-Prussian blue method [7] for acid mucopolysaccharides.[8] The thickened intima and the intimal plaques contained an abundance of blue-staining material, presumably mucopolysaccharide (Fig. 9). Usually this material had a fibrillary appearance and was closely associated with the intimal

Fig. 9.—Abdominal aorta showing abundance of mucopolysaccharide in the intima. Iron-Prussian blue; × 160.

connective tissue cells. In some instances the material appeared amorphous or even granular. The smaller plaques consisted entirely of this material. In the larger plaques the deeper portions generally contained wavy collagenous fibers, usually arranged transversely. Collagenous fibers appeared to be replacing fibrillary mucopolysaccharide material. Some of the larger plaques were composed approximately of half mucopolysaccharide in the inner portions and half collagenous substance in the outer portions. In some plaques the collagenous fibers were more diffusely arranged and were delicate and fibrillar. Where the internal elastic membrane was degenerating or absent, both collagenous and mucopolysaccharide material often extruded from the intimal plaque into the adjacent inner media.

Media.—Lesions of the medial layer were observed in all 12 dogs of the younger group and in 10 of the 14 older dogs. In both groups these were seen

8. The staining of these sections was carried out by Mr. S. K. Abul-Haj.

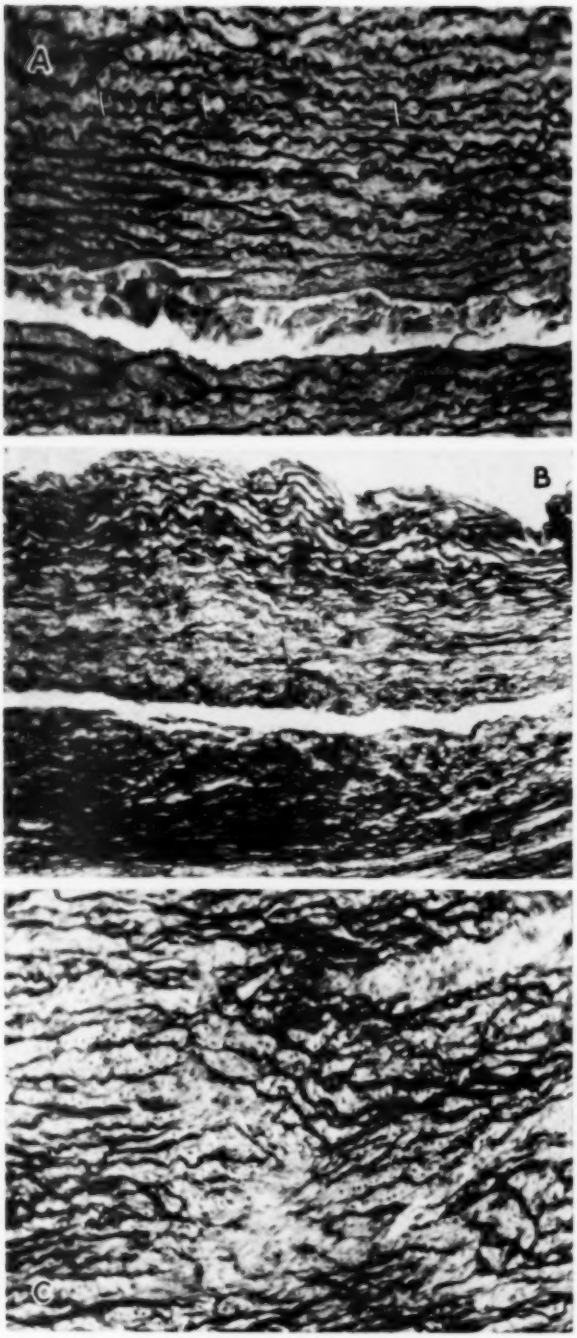

Fig. 10.—*A*, thoracic aorta showing large collagenous deposit in the media. Weigert-Van Gieson stain; × 160.

B, thoracic aorta showing cystic cleft of media and condensation of elastic fibers in outer media. Weigert-Van Gieson stain; × 80.

C, thoracic aorta showing focal grouping of medial smooth muscle cells. Weigert-Van Gieson stain; × 160.

about twice as frequently in the thoracic as in the abdominal segment of the aorta. In the dogs in the younger group the medial alteration was characterized by apparent edema, which often separated muscular and elastic fibers. While occasionally diffuse, this edema was more often focal in nature and in some areas appeared to have been followed by collagenous deposits (Fig. 10*A*). The col-

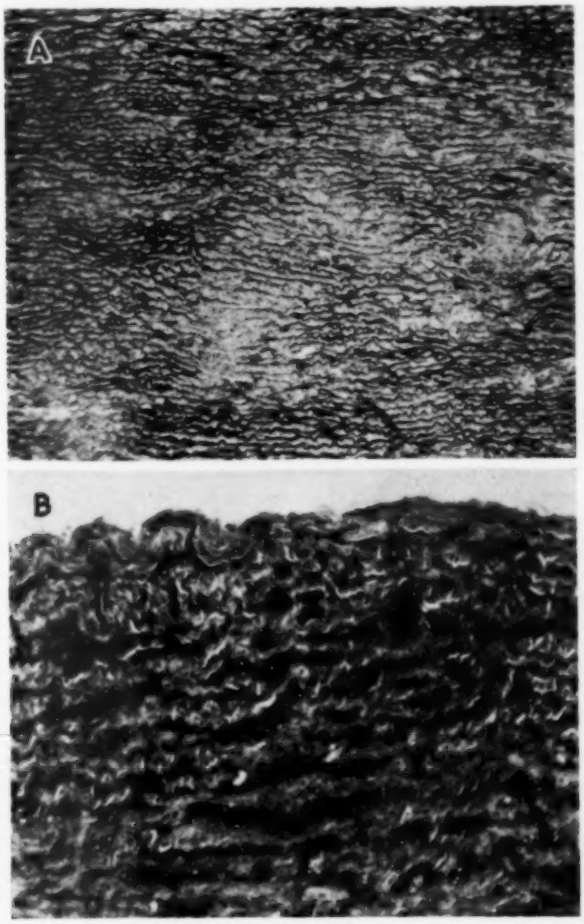

Fig. 11.—*A*, thoracic aorta showing focal loss of medial elastic tissue. Weigert-Van Gieson stain; × 80.
B, thoracic aorta showing deposits of mucopolysaccharide in media. Iron-Prussian blue; × 160.

lagenous scarring was frequently quite pronounced. Cystic lesions, due to edematous separation of muscular and elastic fibers, were not infrequently observed (Fig. 10*B*). In many vessels there was an irregular grouping of smooth muscular cells. These appeared to be hyperplastic and actually proliferating in a focal man-

ner (Fig. 10C). As a rule, the edematous and collagenous changes were most severe in the outer portions of the media, and it seemed apparent that these changes were most pronounced in areas where intimal disease was severe.

The Weigert-Van Gieson stain clearly showed alteration of the elastic tissue, especially where edema, deposits of collagen, or focal groups of smooth muscle cells were present. Usually the elastic tissue was fragmented or absent in these zones (Fig. 11*A*). Surrounding them, the elastic fibers were often coarser, reduplicated, or split and frayed. Condensation of elastic fibers in the outer medial segments was frequently seen (Fig. 10*B*).

In the media of some animals a diffuse interfiber edema was accompanied by a diffuse infiltrate of delicate collagenous fibers. In a few instances, edema of the inner portions of the media had elevated the endothelium without associated intimal fibrosis. Often thinning or loss of medial elastic tissue was not readily correlated with extensive medial edema.

The medial layer contained variable amounts of mucopolysaccharide which, as a rule, was most abundant in the inner portion of the vascular wall (Fig. 11*B*). In part, this material was intracellular, involving both the muscular and the connective tissue cells of the medial layer. Within the cells it appeared as delicate vesicular material. Most of the substances were extracellular and lay between and on the surfaces of the elastic lamellae. In several aortas there were cystic clefts in the outer medial layers (Fig. 10*B*), and these spaces were often filled with considerable amounts of fibrillary, bubbly polysaccharide material. In some areas this substance apparently was being replaced by collagenous fibers, and in two dogs which showed an abundance of medial mucopolysaccharide there were wide collagenous bands throughout the outer medial layers.

Duplicate paraffin sections of some of these aortas were incubated with testicular hyaluronidase and then stained by the iron-Prussian blue method. The mucopolysaccharide in the intima was largely removed by the enzyme treatment. Such treatment, however, removed only small amounts of the medial polysaccharide.

COMMENT

The earliest microscopic evidence of aortic disease in the dog is an alteration of the internal elastic lamina involving reduplication, apparent splitting, or fragmentation. This alteration of the internal elastic membrane is a consistent feature of the intimal plaque in dogs. Since the visible lesions in canine aortas are most numerous at points or areas of stress, it is possible that this elastic alteration is traumatic in origin. Factors such as aging or nutrition, however, obviously cannot be dismissed etiologically, since the incidence of arterial disease of dogs increases with age.

The earliest abnormality in the intima consists of fibroblastic proliferation associated with deposition of a mucoid ground substance. It should be emphasized here that lipids are not demonstrable in the lesion at this stage. The intimal fibroblasts, first arranged irregularly, now assume a perpendicular position. Still later, these cells and their fibers become circumferential in form. This cellular rearrangement is very likely the result of hydrostatic pressure from within the lumen.

The layering often seen in larger intimal plaques must result from successive episodes of fibroblastic proliferation with deposition of mucoid ground substance followed by collagen formation.

Meyer[9] had reviewed the evidence suggesting that proliferating fibroblasts secrete two mucopolysaccharides (hyaluronic acid and chondroitin sulfate) and a collagen precursor. Local acidification results in denaturation of the precursor by the polysaccharide. The chondroitin sulfates then become firmly bound into mature, insoluble, collagenous fibers, while the hyaluronidate is removed enzymatically. The replacing of the mucoid substance with well-formed collagen fibers is a distinct feature of the development of the intimal plaque in the dog. A similar decrease of the metachromatic ground substance of granulation tissue occurs as the collagenous elements mature. That the canine intimal plaque grows from the surface adjacent to the lumen is shown in the observation that the rearrangement of fibroblasts and the collagenous differentiation and replacement of mucoid ground substance progress from the inner portion to the outer.

A later feature of the development of the canine intimal plaque is the elaboration of elastic fibers from the undifferentiated ground substance. When first visible, these elastic structures are delicate fibrils closely surrounding the fibroblasts. As the plaque enlarges, these fibers become coarser and more numerous. They may even reach the proportions of the intimal elastic or medial elastic membranes. In some instances, degenerative changes in the newly formed elastic fibrils supervene.

Hyaline condensation of the intercellular collagen may now occur in some plaques. At times this tissue appears cartilaginous. Pericellular calcific deposits were observed in the intima in only one dog, and were sparse.

It is clear that lipids are not involved in the early aortic disease of the dog. Although extensive intimal fibrosis, even with plaque formation, occurs, lipid is usually absent. In the dog, lipid infiltration of the aorta appears to be a late phenomenon, occurring only in the deeper and older portions of the larger plaques and being, even there, of only minor significance. That the minimal lipid infiltration is not related to cholesterol deposition is shown by the absence of refractile material in plaques containing other lipid substances.

Recently, Steiner and his group[10] described atherosclerosis and arteriosclerosis which they produced in the dog by feeding thiouracil and cholesterol. The arterial lesions so produced were characterized by an intimal collection of cholesterol-containing foam cells associated at times with fibrosis, hyalinization, or calcification. These lesions were identical with those which have been produced in other species by the feeding of cholesterol and were but a part of a widespread deposition of cholesterol in many tissues.[11] *It should be noted that this is an artificial arterial disease which has little resemblance to the spontaneous canine arteriosclerosis described here.* Indeed, it is likely that the pathogenesis of the cholesterol-induced lesion in the dog is distinctly different from that of the spontaneous disease.

Some of the arteriosclerotic lesions described by these authors may have been spontaneous intimal lesions that were modified by later accumulation of lipids, including cholesterol. Such secondary lipid infiltration of preexisting fibrous

9. Meyer, K.: Am. J. Med. **1**:676, 1946; Physiol. Rev. **27**:335, 1947; Science **113**:596, 1951.

10. Steiner, A., and Kendall, F. E.: Atherosclerosis and Arteriosclerosis in Dogs Following Ingestion of Cholesterol and Thiouracil, Arch. Path. **42**:433 (Oct.) 1946. Steiner, A.; Kendall, F. E., and Bevans, M.: Am. Heart J. **38**:34, 1949.

11. Kuntz, A., and Sulkin, N. M.: Lesions Induced in Rabbits by Cholesterol Feeding, with Special Reference to Their Origin, Arch. Path. **47**:248 (March) 1949.

plaques has been shown to occur in the abdominal aorta of birds, spontaneously as well as experimentally.[12] Furthermore, recent studies by Moon [13] reveal that the initial lesion of coronary arteriosclerosis of man also consists of intimal fibrosis and that lipid infiltration is a late complication in larger, older plaques.

Progressive enlargement of the spontaneously occurring intimal plaque has several secondary effects. The intimal elastic membrane is compressed and distorted by the proliferating intima, and further disruption, fragmentation, and degeneration may result. The medial layer beneath may also be compressed, and its elastica condensed and coarsened.

Alteration of the medial layer has a certain resemblance to that of the intima. Deposition of considerable amounts of mucoid ground substance occurs and, while diffuse, is usually most evident in the inner portion of the medial layer. In the dog, medial disease is characterized by (1) collagenous replacement, (2) focal degeneration or loss of elastic tissue, and (3) focal proliferation of smooth muscle cells. Cyst formation, with deposits of acid mucopolysaccharide and collagen, occurs frequently. That aging is a factor in the causation of this medial disease is suggested by the absence of the disease in young dogs [6] and by its resemblance to medial disease of the human being.[14]

Most previous reports on arteriosclerosis of the dog have not included studies of the coronary arteries and the heart, although chronic cardiac disease and even sudden death from cardiac failure are not uncommon in old dogs. Our studies have shown a variety of lesions of the coronary arterial system, some of which bear a remarkable resemblance to coronary arteriosclerosis of the human being. The secondary lesions of the myocardium, which include infarction and myocardial fibrosis, readily explain the cardiac disability observed in old dogs.

SUMMARY

The present report is based on a study of 26 dogs, 14 of which were 8 to 17 years of age.

The incidence of spontaneous arteriosclerosis of the aorta is high in dogs. The majority of the older dogs showed evidence of cardiac disease, including coronary arteriosclerosis and myocardial infarction or fibrosis.

The outstanding spontaneous lesion of canine arteriosclerosis is intimal fibrosis associated with deposition of a mucoid ground substance and collagen. Alteration of the internal elastic membrane may occur without intimal disease, and is almost invariably observed when intimal thickening is present.

Medial disease of the aorta was observed in the majority of dogs and was characterized by deposition of mucoid substances and collagen, often associated with focal proliferation of smooth muscle cells and focal alteration of elastic tissue.

Lipids appeared to play no part in the pathogenesis of the earliest intimal lesions. Although lipids were present in later lesions, lipid infiltration appeared to be a minor process even in the arteriosclerotic plaques of older dogs.

Cholesterol was not found in older lesions even where other lipids had infiltrated the intimal fibrous plaques.

12. Chaikoff, I. L.; Lindsay, S.; Lorenz, F. W., and Entenman, C.: J. Exper. Med. **88**:373, 1948.

13. Moon, H. D.: Personal communication to the authors.

14. Rottino, A.: Medical Degeneration of the Aorta: A Study of Two Hundred and Ten Routine Autopsy Specimens by a Serial Block Method, Arch. Path. **28**:377 (Sept.) 1939.

METHYLCHOLANTHRENE-INDUCED TUMORS IN GUINEA PIGS

With a Review of the Literature on Tumors Induced with Polycyclic Carcinogenic Hydrocarbons in This Species

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BECAUSE of difficulties observed in inducing tumors in guinea pigs with carcinogenic substances, these animals have not been used so frequently in cancer research as rats and mice. The relatively poor response of guinea pigs to carcinogenic stimulation has been attributed to an inherent species resistance. Supportive evidence for this conclusion is found in the repeated observation that spontaneous neoplasms are remarkably less frequent in guinea pigs than in most other laboratory animals. In a review of the literature in 1937, Leader [1] found only 21 cases of spontaneous tumors occurring in guinea pigs. In three inbred families of guinea pigs at the Beltsville Agricultural Station of the United States Department of Agriculture no neoplasms have been observed in 15,000 animals.[2] This is a remarkable observation considering the well known frequency of spontaneous tumors in rats and mice.

It has been possible, however, to induce tumors in guinea pigs regularly by means of a high concentration of methylcholanthrene, as reported by Shimkin and Mider [3] and Esmarch.[4] Guinea pigs have singular usefulness in experimental work, because scurvy develops in these animals, as in man and higher apes, when ascorbic acid is removed from the diet. Rats, mice, rabbits, or birds can synthesize ascorbic acid and therefore are not susceptible to scurvy.

This study was aided by a grant from the John and Mary R. Markle Foundation.

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From the Department of Pathology of the Washington University School of Medicine, St. Louis, and the Department of Pathology of The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

1. Leader, S. A.: Osteogenic Sarcoma of Femur in Guinea-Pig, Am. J. Cancer **29**:546-550, 1937.

2. Wright, S., and Eaton, O. N.: Persistence of Differentiation Among Inbred Families of Guinea Pigs, Technical Bulletin 103, U. S. Department of Agriculture, 1929.

3. Shimkin, M. B., and Mider, G. B.: Induction of Tumors in Guinea Pigs with Subcutaneously Injected Methylcholanthrene, J. Nat. Cancer Inst. **1**:707-725, 1941.

4. Esmarch, O.: The Guinea Pig as an Experimental Animal in Cancer Research, Acta path. et microbiol. scandinav. **19**:100-107, 1942.

The studies reported here concern a pathologic study of neoplasms produced in guinea pigs by the subcutaneous injection of methylcholanthrene dissolved in sesame oil after the technique of Shimkin and Mider.[5] For a more complete evaluation of the response of guinea pigs to carcinogenic stimulation, all of the reports of tumors that have been induced in this species with polycyclic aromatic hydrocarbons have been collected from the literature for review.

Neoplasms so induced were invariably some type of sarcoma. This observation suggested the possibility of influencing the development of the tumors in guinea pigs by producing scurvy during the induction period, because of the well known effect of ascorbic acid deficiency on extracellular substances produced by mesenchymal cells. The effect of scurvy on the induction of tumors will be the subject of another communication.[5]

EXPERIMENTAL PROCEDURE

Ninety-eight young but fully matured male and female guinea pigs, averaging 300 gm. in weight, were purchased from commercial breeders. The methylcholanthrene [6] was dissolved in sesame oil to a concentration of 10 mg. per cubic centimeter. Each animal was given 30 mg. of methylcholanthrene in 10-mg. doses injected into the subcutaneous tissues of the axilla of the right foreleg at two-week intervals. The animals were maintained on a commercial diet,[7] with daily feedings of lettuce.

TABLE 1.—*Incidence of Tumors and Their Classification*

Animals surviving to the tumor-bearing period	73
Animals with tumors	39
Animals dying without tumors after 170 days	34
Tumors classified as fibrosarcoma	17
Tumors classified as osteosarcoma	10
Tumors classified as liposarcoma	12

Necropsy and Histologic Technique.—The animals were examined at weekly intervals for the development of tumors. Guinea pigs in which tumors developed were removed from the group and placed in a separate cage for special observation. Each tumor was allowed to grow until the animal died or became moribund. After death a complete necropsy was performed, and representative pieces of the tumor tissue were fixed in Zenker's fluid. The whole animal with the tumor was fixed in neutral 4% formaldehyde (N. F. formaldehyde 1:10). Hematoxylin and eosin and Mallory's phosphotungstic acid-hematoxylin stains were used routinely. The Sudan III stain for fat was used in those tumors suspected of being liposarcomas.

RESULTS

In 39 of the 73 guinea pigs that survived the appearance of the first tumor (170 days) a tumor developed in the subcutaneous tissues of the right axilla where the methylcholanthrene had been injected.

The experiment was terminated after 588 days, since by this time all animals except two either had a tumor or had died. The incidence and the histologic classification of the tumors produced are given in Table 1. Pertinent data concerned with the histologic types of neoplasm observed in these experiments are given in Table 2.

5. Russell, W. O.; Ortega, L. R., and Wynne, E. S.: The Effect of Scurvy on the Induction of Tumors by Methylcholanthrene in Guinea Pigs, Cancer Res., to be published.

6. This was obtained from the Eastman Kodak Company.

7. This was "rabbit chow checkers," obtained from the Ralston Purina Company.

TABLE 2.—*Induction Times for the Three Types of Sarcoma*

Guinea Pig	Sex	Induction Period	Type of Tumor	Description of Tumor
A. Seventeen Tumors Composed of Neoplastic Fibroblasts. Average Induction Time 306 Days				
48D*	M	183	Fibrosarcoma	Rapidly growing, infiltrating, anaplastic
19I	M	183	Fibrosarcoma	Slowly growing, encapsulated, well differentiated
61I	F	215	Fibrosarcoma	Rapidly growing, anaplastic, extensive necrosis
66I	F	230	Fibrosarcoma	Slowly growing, encapsulated, well differentiated
56D	M	232	Fibrosarcoma	Moderately rapidly growing, encapsulated, presenting foci of anaplasia
38N	M	284	Fibrosarcoma	Slowly growing, encapsulated
61D	F	285	Fibrosarcoma	Moderately rapidly growing, well differentiated
65N	M	290	Fibrosarcoma	Rapidly growing, infiltrating, anaplastic
68N	F	290	Fibrosarcoma	Rapidly growing, poorly differentiated
56I	M	313	Fibrosarcoma	Slowly growing, encapsulated
68N	F	335	Fibrosarcoma	Rapidly growing, infiltrating anaplastic
37D	M	340	Fibrosarcoma	Rapidly growing, poorly differentiated, highly anaplastic
8N	M	340	Fibrosarcoma	Rapidly growing, infiltrating, anaplastic
5N	M	354	Fibrosarcoma	Moderately rapidly growing, well differentiated, encapsulated, foci of anaplasia
66N	F	371	Fibrosarcoma	Moderately rapidly growing, encapsulated
67I	F	459	Fibrosarcoma	Rapidly growing, encapsulated, anaplastic
36I	M	490	Fibrosarcoma	Rapidly growing, infiltrating, highly anaplastic
B. Ten Tumors Containing Bone or Osteoid Tissue. Average Induction Time 256.4 Days				
2D	F	174	Osteosarcoma	Moderately rapidly growing, presenting much osteoid tissue
15I	M	175	Osteosarcoma	Moderately rapidly growing, presenting osteoid tissue and bone
33D	M	177	Osteosarcoma	Rapidly growing, presenting osteoid tissue
51D	M	177	Osteosarcoma	Moderately rapidly growing, predominantly fibrosarcoma with foci of osteoid tissue
16I	M	179	Osteosarcoma	Rapidly growing, presenting much osteoid tissue, classifiable as fibrosarcoma
46D	F	217	Osteosarcoma	Rapidly growing, anaplastic, presenting osteoid tissue and bone
62I	M	218	Osteosarcoma	Moderately rapidly growing, predominantly fibrosarcoma with foci of osteoid tissue
19D	F	336	Osteosarcoma	Moderately rapidly growing, encapsulated, presenting osteoid tissue and bone
71N	F	368	Osteosarcoma	Rapidly growing, anaplastic, presenting osteoid tissue
70N	M	543	Osteosarcoma	Slowly growing, presenting osteoid tissue
C. Twelve Tumors Containing Lipoblasts. Average Induction Time 350.3 Days				
30D	F	170	Liposarcoma	Rapidly growing, poorly differentiated, encapsulated
6D	M	180	Liposarcoma	Rapidly growing, showing poorly differentiated lipoblasts, encapsulated
50D	M	205	Liposarcoma	Slowly growing, encapsulated, well differentiated
12D	M	275	Liposarcoma	Slowly growing, encapsulated
15D	F	310	Liposarcoma	Slowly growing, encapsulated, showing well-differentiated lipoblasts
63I	F	333	Liposarcoma	Rapidly growing, encapsulated, showing marked anaplasia
11I	F	353	Liposarcoma	Slowly growing, encapsulated, presenting highly differentiated lipoblasts
34D	M	368	Liposarcoma	Moderately rapidly growing, predominantly characteristic fibrosarcoma
2N	M	380	Liposarcoma	Rapidly growing, encapsulated, anaplastic
52I	M	479	Liposarcoma	Slowly growing, encapsulated, presenting well-differentiated lipoblasts
69N	M	563	Liposarcoma	Moderately rapidly growing; a fibrosarcoma with foci of liposarcoma
21N	F	588	Liposarcoma	Slowly growing, encapsulated, well differentiated

* The letter D following the number of the guinea pig indicates the animal was given periods of scurvy throughout the experiment; C, normal control diet; I, inanition diet. The details of the diets and of the procedure are reported elsewhere.[5]

PATHOLOGIC STUDY OF THE TUMORS

All of the tumors were classified as one or another type of a malignant mesenchymal tumor. Seventeen tumors were classified as fibrosarcoma, 10 tumors produced osteoid tissue or bone, and 12 were classified as liposarcoma. In 5 of the

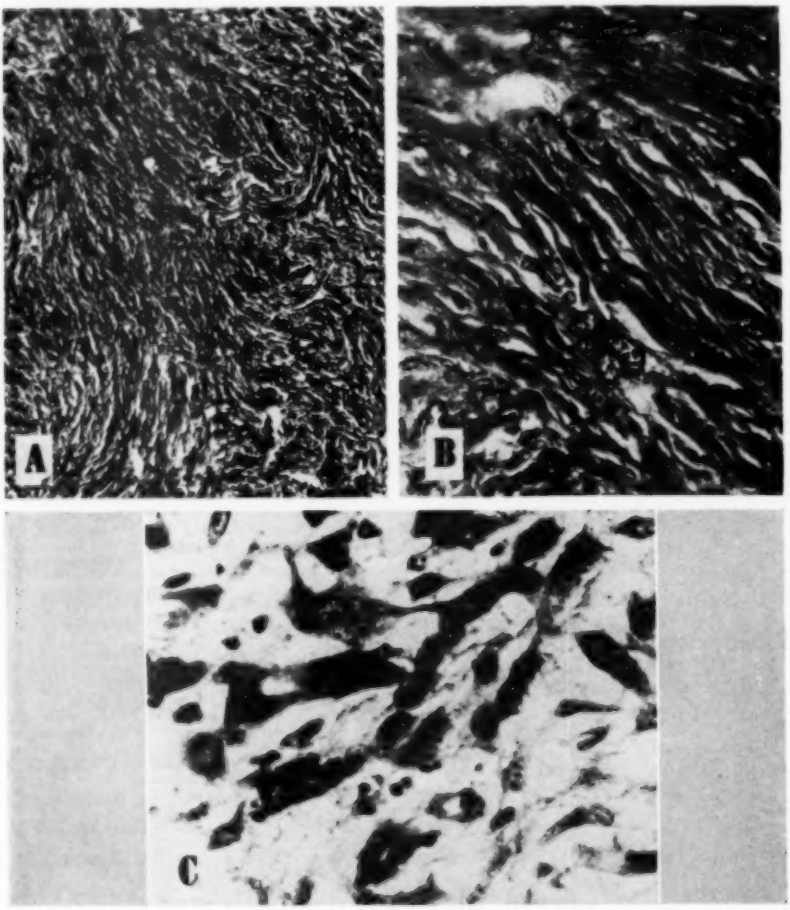

Fig. 1.—Types of fibrosarcoma: *A*, fibrosarcoma showing closely packed spindle-shaped cells forming interlacing, parallel patterns. Hematoxylin-eosin; × 125.

B, higher power magnification of *A*. Fibroblastic tumor cells are seen with abundant intercellular collagen. Note the conspicuous variation in the size of the nuclei in the lower center part of the field; × 464.

C, rapidly growing anaplastic sarcoma showing marked anisonucleosis. The cells have a syncytial arrangement, with a loose, edematous-appearing intercellular matrix. Anisocytosis and anisonucleosis are prominent. A mitotic figure is seen in the lower left side of the field. Hematoxylin-eosin; × 464.

tumors two types of growth were noted, but in all of these there was a fibrosarcoma component in the growth. The tumors are collectively considered under the following three principal histologic types of tumor observed.

Fibrosarcoma (Guinea pigs 48-D, 19-I, 61-I, 66-I, 56-D, 38-N, 41-D, 65-N, 68-N male, 68-N female, 58-I, 37-D, 8-N, 5-N, 60-N, 67-I, 36-I).—All of these tumors were composed of cells resembling fibroblasts showing varying degrees of differentiation. The tumors arising at the sites of injection of the carcinogen varied in gross appearance from a firm, well-encapsulated tumor to a soft, fluctuant, nonencapsulated, invasive tumor. Animals having typical examples of these two types are described below in detail.

(*a*) Slowly Growing, Well-Differentiated Fibrosarcoma (Guinea Pig 19-I): A tumor was first noticed, after 183 days, in the right axilla at the site of the injection of the carcinogen. When first observed the nodule was 1.5 cm. in diameter. After a four-month period the diameter had increased to 3.5 cm., at which time the animal was killed.

At necropsy the tumor was identified as an encapsulated mass of firm tissue situated in the right axilla, unattached to the thorax and the overlying skin. On sectioning, the tissue was firm, grayish-pink and white, and had a fibrillar appearance. No other tumor was demonstrable in the regional lymph nodes or in the abdominal and thoracic viscera.

Microscopic study disclosed a moderately cellular tumor. The cells were elongated, spindle-shaped, closely packed, and frequently arranged in parallel formation (Fig. 1*A* and *B*). Mitotic figures were noted but were infrequent. No neoplastic giant cells were seen. A phosphotungstic acid-hematoxylin stain of the tumor revealed an abundant brownish-red-staining intercellular collagen and fine blue-staining intracellular fibrils. The capsule of the tumor was composed of bundles of connective tissue arranged in parallel.

(*b*) Rapidly Growing, Poorly Differentiated Anaplastic Fibrosarcoma (Guinea Pig 37-D): This tumor was first observed, after 340 days, as a soft nodule 1 cm. in diameter in the axilla. Because the tumor was so soft, its identity as a true neoplasm at first was questioned. The swelling rapidly increased in size so that in two weeks it was 7 cm. in diameter and seriously interfered with the normal gait of the animal. At that time the possibility of an acute pyogenic abscess was considered, since the swelling was soft, fluctuant, and did not resemble the usual form of swelling seen with the other tumors. An attempt to aspirate the lesion with a large-bore aspirating needle was unsuccessful; therefore, a small scalpel incision was made in the mass, and soft, semifluid, red hemorrhagic material was obtained. No tissue of definite form could be identified.

Pressure on the mass resulted in a nearly complete evacuation of the material contained in it. There remained only a deflated sac of tissue about 20% of the size of the original mass. The mass reformed its original bulk in approximately two weeks, at which time the same type of soft hemorrhagic material was evacuated. Following the second evacuation, secondary infection developed, with purulent fluid discharging from the wound, and death occurred in four days.

Necropsy disclosed, in the subcutaneous tissue of the right axilla, a cystic mass, partially filled with pus and brownish-red, soft, necrotic tissue. The wall of the cavity was lined by a 1-cm. zone of soft, deep reddish-brown tissue surrounded by a fairly well-defined fibrous capsule. The thoracic and abdominal organs disclosed no significant pathologic change. No metastatic tumor was found in the regional lymph nodes or in the parenchymatous organs.

Microscopic sections of the hemorrhagic material originally evacuated from the mass and of the wall of the cysts were taken at necropsy and showed the same type of tumor tissue. This was highly cellular, with many small proliferating capillaries. A variety of cellular types was noted. The most prominent cell was a round or oval cell, frequently having one or two short processes. The nuclei, which were moderately chromatic and frequently elongated, possessed prominent nucleoli and chromatin, heavily concentrated at the nuclear membrane. In scattered areas the protoplasmic extensions of the cells were more prominent, causing the cells to appear as short plump fibroblasts (Fig. 1*C*). Numerous large neoplastic giant cells with multiple nuclei were found. Mitotic figures were present in all of the several types of cells, and atypical mitotic figures were present in the neoplastic giant cells. A phosphotungstic acid-hematoxylin stain of

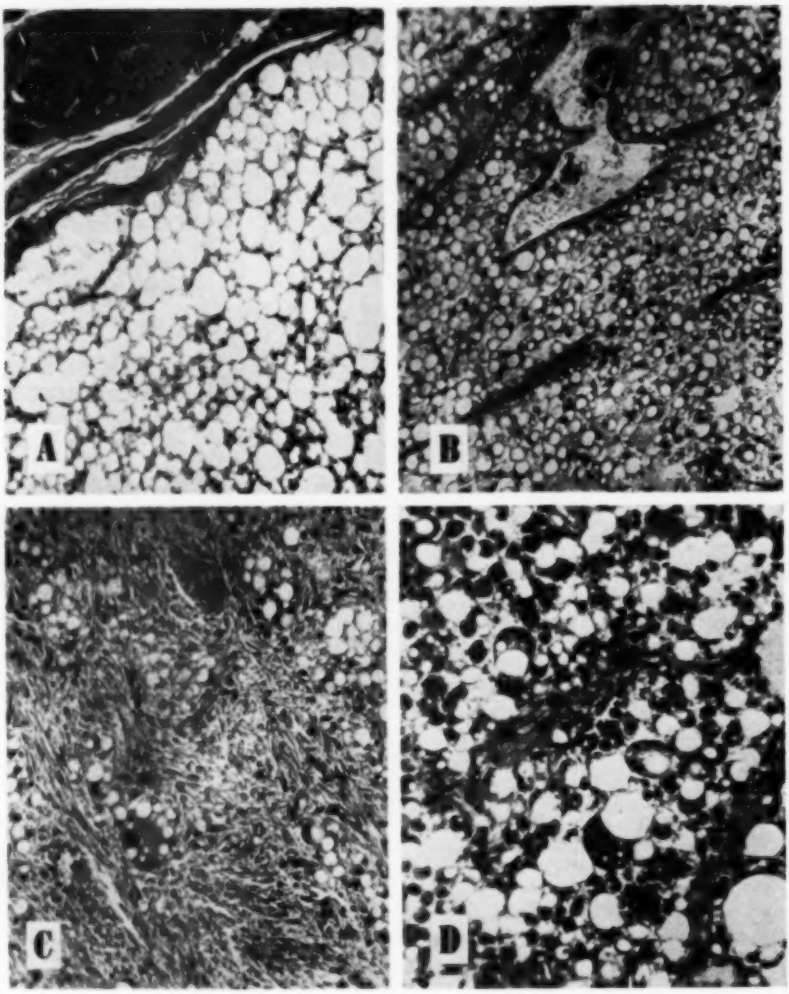

Fig. 2.—Types of liposarcoma: *A*, liposarcoma composed of well-differentiated lipoblasts. Note the capsule shown in the upper left part of the field. The variation in the size of the cells, with visible anisonucleosis, identifies the fatty tissue as neoplastic. Hematoxylin-eosin; × approx. 70.

B, liposarcoma less well-differentiated than the tumor shown in *A*. The lipoblasts have conspicuous variation in the size of the fat vacuole. A large, thin-walled blood space is seen in the upper central part of the field. Hematoxylin-eosin; × 139.5.

C, liposarcoma containing a mixed component of fibrosarcoma. The fibroblastic cells surround neoplastic fat cells. Giant cells are noted in the lower left part of the field. Hematoxylin-eosin; × 209.

D, tumor with marked variation in the lipoid content of the cells. (Compare with *A*, *B*, and *C*.) Some cells have multiple vacuoles of varying size, while others have a homogeneous mass of basophilic-staining cytoplasm, with extremely small vacuoles giving a granular appearance to the cytoplasm. Hematoxylin-eosin; × 418.6.

the tumor showed a small amount of brown-staining collagen between the cells in those areas where the cells resembled fibroblasts. These cells occasionally contained some blue-staining intracellular fibrils.

Liposarcoma (Guinea Pigs 30-D, 6-D, 50-D, 12-D, 15-D, 63-I, 11-I, 2-N, 52-I, 21-N, 34-D, and 69-N).—See Figure *2B* for Guinea Pig 6-D, Figure *2C* for Guinea Pig 34-D, and Figure *2D* for Guinea Pig 63-I.

All of these tumors were identified as liposarcoma. The tumors showed varying degrees of differentiation from a slowly growing mass of neoplastic fat cells to a more rapidly growing tumor composed of fibroblastic-appearing cells, with only an occasional cell containing vacuoles of fat. Two protocols representative of these two types are given below.

(*a*) Well-Differentiated, Slowly Growing Liposarcoma (Guinea Pig 52-I): This tumor, discovered after an experimental period of 479 days, was an unusually firm, hard mass in the right axilla, measuring approximately 5 mm. in diameter. It could be distinguished from the enlarged lymph nodes by its unusual firmness. The mass was carefully observed for two months, when it measured 1.5 cm. in diameter. After four months the tumor was 2.5 cm. in diameter, at which time the animal was killed.

At necropsy the tumor was observed to be an encapsulated firm mass of grayish-pink tissue in the right axilla, measuring 2 cm. in diameter and not attached to the skin or the underlying structures. After sectioning, a soft, homogeneous-appearing, yellowish-pink tissue was seen. No tumor or other significant pathologic change was found in the regional lymph nodes or in the viscera of the abdomen and the thorax. Microscopic examination of the tumor showed well-differentiated fat cells (Fig. 2*A*). The cells contained a single oval vacuole that nearly replaced the cytoplasm of the cell and displaced the nucleus to one side. In a few cells two vacuoles were seen in the cytoplasm. The nucleus was small, moderately chromatic, and occasionally contained a small nucleolus. No mitotic figures were noted in the several sections. A small amount of connective tissue stroma and small blood vessels accompanied the tumor. A well-developed fibrous tissue capsule containing scattered lymphocytes and plasma cells surrounded the tumor. A part of the tumor fixed in formaldehyde was stained with Sudan III. The vacuoles were sudanophilic.

(*b*) Rapidly Growing, Poorly Differentiated Liposarcoma (Guinea Pig 63-I). After an experimental period of 333 days, this tumor appeared as a firm nodule, 1 cm. in diameter, in the right axilla. By two months it had increased to a diameter of 8 cm. The animal, having lost considerable weight, was killed at this time.

Necropsy revealed a 7-cm. subcutaneous mass in the right axilla, which was finely lobulated and grayish-pink. Scattered small red foci of necrosis were noted. Microscopic sections revealed a highly cellular tumor, composed of oval or slightly stellate cells. These cells had basophilic-staining cytoplasm that frequently contained vacuoles of varying size and shape. Mitotic figures were numerous, and occasionally large giant cells with irregularly shaped chromophilic nuclei were seen. In some areas the cells did not contain any vacuoles. Sudan III stains demonstrated fat in the vacuoles.

Osteosarcoma (Guinea Pigs 2-D, 15-I, 33-D, 46-D, 19-D, 71-N, 70-N, 51-D, 16-I, 62-I).—See Figure 3*A* for Guinea Pig 2-D and Figure 3*B* for Guinea Pig 19-D.

The term "osteosarcoma" has been used to designate those tumors in which there was production of osteoid tissue or bone. In several of these tumors the osteoid tissue was an inconspicuous part of the total mass and was found only after several sections were taken. However, these tumors exhibited more gross and microscopic evidence of a malignant tumor than did the liposarcomas or the fibrosarcomas. The following protocol is illustrative of this type of neoplasm.

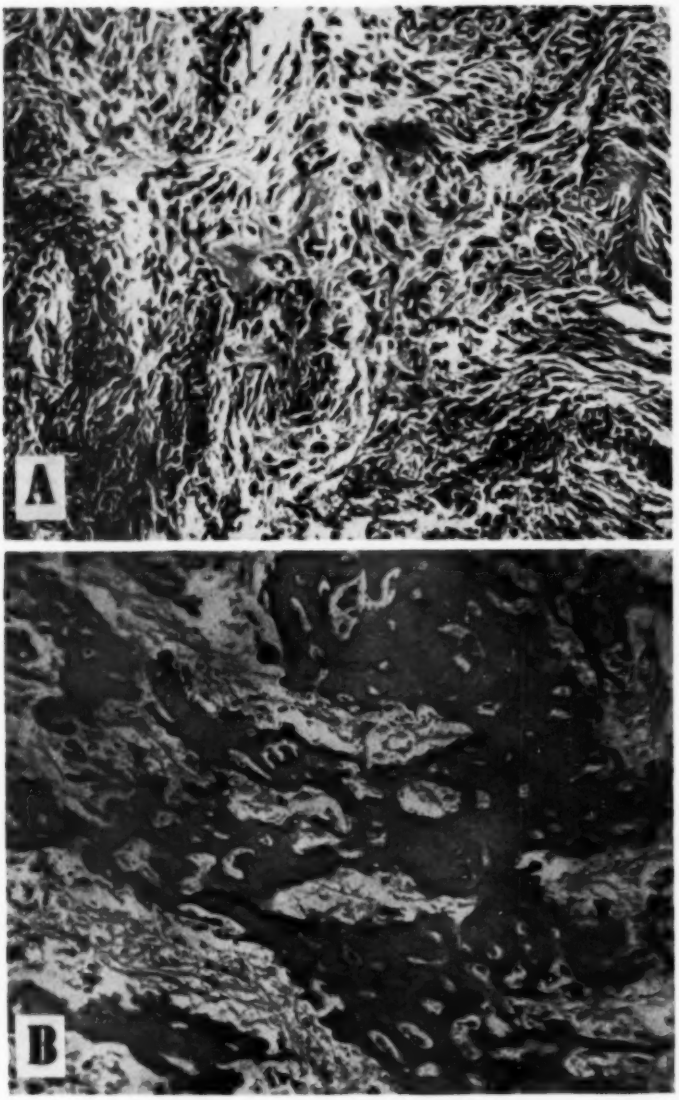

Fig. 3.—Types of osteosarcoma: *A*, osteosarcoma showing typical stellate-shaped osteoblasts. The amount of intercellular substance is scant. However, the arrangement of cells relative to intercellular substance is characteristic of neoplastic osteoid tissue. Hematoxylin-eosin; × 200.

B, osteosarcoma showing high differentiation with a mosaic pattern of neoplastic bone, accompanied by a loose type of connective tissue stroma. A small amount of calcification is present in the trabeculae. Compare with *A*. Hematoxylin-eosin; × 200.

(*a*) Rapidly Growing, Highly Anaplastic Osteosarcoma (Guinea Pig 46-D): This tumor appeared, after an experimental period of 217 days in the right axilla as a firm, hard nodule 1 cm. in diameter. By three weeks it had increased to a diameter of 3 cm. and was firmly attached to the underlying tissues. During the next two months three biopsies were done, the third one being fatal to the animal. At the time of death the mass measured 7 cm. in diameter.

Necropsy revealed an irregularly lobulated mass of firm, grayish-red tissue that was firmly attached to the lateral thoracic wall. There was no demonstrable line of cleavage around the tumor's deep attachments, but superficially the mass was attached to the overlying skin in the region of the previously taken biopsy specimens. One lymph node, 1 cm. in diameter, was recovered from the axilla. Section revealed a firm, gray-pink, homogeneous tissue replacing the characteristic tissue of the lymph node. An irregularly mottled gray, red, and pink surface was seen in sections through the main mass of the tumor. This tissue was cut with great difficulty. Numerous large foci of firm gritty tissue, having a lighter color than the other, softer tissues, were identified on the cut surface. No other pathologic change or tumor was identified in the thoracic and abdominal viscera.

Microscopic sections of the large tumor revealed several different types of growth. In the areas of softer tissues the tumor was composed for the most part of elongated cells resembling fibroblasts showing no special orientation. Intermingled with these cells were polyhedral-shaped cells, frequently showing a tendency to develop short, blunt processes. Large numbers of neoplastic giant cells, frequently containing multiple bizarre-shaped nuclei and atypical mitotic figures, were present. In the areas of firm tumor, which had the gritty surface, there was an entirely different type of growth. In these places the tumor was forming scattered well-defined masses of osteoid tissue, and in many places small spicules of calcified bone were identified. The intervening tissue between the foci of osteoid and bony tissue was essentially of the same type as that described in the softer parts of the tumor.

EFFECTS OF THE CARCINOGEN

Shimkin and Mider [3] observed a characteristic syndrome in a significant number of guinea pigs which was believed to be a generalized systemic toxic effect of the methylcholanthrene administered. The syndrome was similar in all of the animals and consisted of massive ascites, atrophy of the spleen, hemorrhage in the liver, and death. This same syndrome was noticed in 10 of our animals. The initial symptoms of the disease were loss of weight, inactivity of these as compared with the other animals, extreme irritability when handled, and death in three days to one week.

In all of the animals the axillary lymph nodes became enlarged on the side of injection. They became palpable in about two months and remained so for the duration of the experiment. For this reason it was difficult to determine accurately when a tumor developed, since progressive enlargement of an axillary mass was necessary to distinguish it from the enlarged lymph nodes.

TRANSPLANTATION OF INDUCED TUMORS

Numerous attempts were made to transplant induced tumors into other guinea pigs. Masses of tumor cells were injected through a large-bore needle into the subcutaneous tissues and the peritoneum. All observed types of tumors were used, namely, liposarcoma, fibrosarcoma, and osteosarcoma. In no instance was there continued progressive growth of a tumor transplant. Survival of the tumor masses for varying periods up to four months was the rule when a highly anaplastic tumor was placed in the subcutaneous tissues. Examination of the transplanted tumors that survived showed a well-defined fibrous tissue capsule surrounding the tumor, with no evidence of tumor extension beyond it (Fig. 4).

REVIEW OF LITERATURE ON TUMORS OF GUINEA PIGS INDUCED WITH POLYCYCLIC HYDROCARBONS

In an extensive survey of the literature in 1941, Hartwell[8] tabulated 362 polycyclic hydrocarbons which have been tested for carcinogenic activity. However, only three of these have been tested in guinea pigs, and all three have been found to cause tumors.[9] Subsequent to Hartwell's[8] survey successful experiments with methylcholanthrene, dibenzanthracene, 9,10-dimethyl-1,2-benzanthracene, and benzpyrene have been collected from the literature. Methylcholanthrene proved the most reliable and efficient carcinogen, since tumors were usually produced in a higher percentage of animals when this substance was used. In our experiments, tumors developed in 39 of 73 animals. The tumors produced by these carcinogens reported in the literature are tabulated in Table 3.

Shimkin and Mider,[1] in 1941, reported experiments with three families of highly inbred guinea pigs obtained from the Beltsville Experiment Station of the United States Department of Agriculture. The animals had been homozygous

Fig. 4.—A homologous transplant of a rapidly growing sarcoma after four months. The neoplastic cells are surrounded by a broad zone of connective tissue forming a well-defined capsule. Areas of necrosis can be seen in the central part. The cells were viable, but there was no evidence of increase in size of the tumor over a four-month period.

since 1915 for such characteristics as color of hair and tendencies toward polydactylism and the production of certain types of monsters.[2] Twenty-one of these inbred guinea pigs were given a single subcutaneous injection of 20 mg. of methylcholanthrene dissolved in 2 cc. of sesame oil. Fourteen inbred guinea pigs received 10-mg. doses of the same carcinogen four times at two-week intervals. Ten stock guinea pigs of unknown genetic constitution received 30 mg. of methylcholanthrene in 3 cc. of sesame oil. Forty guinea pigs were still alive four weeks

8. Hartwell, J. L.: Survey of Compounds Which Have Been Tested for Carcinogenic Activity, Federal Security Agency, U. S. Public Health Service, Bethesda, Md., 1941.

9. (*a*) Athias, M.: Sarcoma of the Heart of a Guinea Pig After Intracerebral Injection of Methylcholanthrene: Question of Spontaneous or Experimental Origin of the Tumor, Compt. rend. Soc. de biol. **126**:585-587, 1937. (*b*) Haagensen, C. D., and Krehbiel, O. F.: Liposarcoma Produced by 1:2-Benzpyrene, Am. J. Cancer **27**:474-484, 1936. (*c*) Schabad, L. M.: Experimental Tumors in Guinea Pigs, Arch. sc. biol. **51**:112-119, 1938. (*d*) Shear, M. J.: Studies in Carcinogenesis: V. Methyl Derivatives of 1:2-Benzanthracene, Am. J. Cancer **33**:499-537, 1938.

TABLE 3.—*Analysis of Tumors Produced in Guinea Pigs with Methylcholanthrene, Dibenzanthracene, Benzanthracene, and Benzpyrene*

Author	Carcinogen	Site of Injection	Animals in Experiment	Animals with Tumor	Sex		Range of Exper. Period, Days	Type of Tumor or Tumors		
					M	F		Fibrosarcoma	Liposarcoma	Osteosarcoma
Haagensen and Krehbiel [9b]	3,4-Benzpyrene	Subcutaneous	13	5	4	1	306-394	4	4	0
Schabad [9c]	1,2,5,6-Dibenzanthracene	Intraperitoneal	7	2	?	?	570-600	1	1	0
Shear [9d]	3,4-Benzpyrene	Subcutaneous	23	2	?	?	420-600	?	?	?
Warren and Gates [12]	3,4-Benzpyrene	Subcutaneous	53	4	?	?	340-579	2	2	0
Shimkin and Mider [8]	Methylcholanthrene	Subcutaneous	40	15	20	9	126-280	17	6	5
Esmarch [4]	Methylcholanthrene	Subcutaneous	10	3	0	3	420-630	3	0	0
Esmarch [4]	Methylcholanthrene	Intraperitoneal	10	4	4	0	300-555	4	0	0
Steiner [10]	Methylcholanthrene	Subcutaneous	25	1	?	?	495	1	0	0
Berenblum [11]	9,10-Dimethyl-1,2-benzanthracene	Subcutaneous	18	7	?	?	990	7	0	0
Russell, Ortega and Wynne [5]	Methylcholanthrene	Subcutaneous	73	39	24	15	170-588	17	10	12
Total			272	82	..	..	.....	56	23	17

after the injections. Thirty-four of the animals survived over 18 weeks, and 29 had tumors at the sites of injection of the carcinogen. The average time for development of the tumors was 25 weeks after the injection, the first tumor being palpable in 18 weeks. In seven of the guinea pigs two separate subcutaneous tumors developed, and in two others the tumors were of a mixed histologic type. Seventeen of the 29 tumors were fibrosarcomas, 7 were liposarcomas, and 5 were osteosarcomas. Pulmonary metastases were noted in four instances. Six tumors were successfully transplanted into guinea pigs of the same family.

Conversely, Athias [9a] reported only one tumor in 33 guinea pigs given methylcholanthrene. Even in this experiment the possibility that the intracerebrally administered carcinogen was related to the tumor that developed in the heart is open to serious question. Esmarch [4] induced seven tumors in 20 guinea pigs by injecting methylcholanthrene. In the first group of 10 animals, which had received a single subcutaneous injection of 10 mg. of glycerol-moistened crystalline methylcholanthrene, three tumors developed. Four animals in the second group of 10, which were given the carcinogen by the intraperitoneal route, had sarcoma within the abdominal cavity after experimental periods ranging from 13 to 18.5 months. One of the tumors was successfully transplanted to other guinea pigs. Cylindric pellets containing 10 mg. of methylcholanthrene plus 10 mg. of cholesterol were implanted in the salivary glands of 25 guinea pigs by Steiner.[10] After an induction period of 16.5 months, one "spindle cell sarcoma" was identified.

Berenblum [11] injected 9,10-dimethyl-1,2-benzanthracene subcutaneously into 30 guinea pigs and succeeded in producing seven tumors. Two of the guinea pigs did not survive the injections. Histologically, the tumors induced in the guinea pigs were predominantly "spindle cell sarcomas," with the pattern varying from that of a well-differentiated fibrosarcoma to that of a highly atypical mixed-cell sarcoma with tumor giant cells, abnormal mitoses, and other evidence of rapid growth.

Haagensen and Krehbiel [9b] reported liposarcomas and fibrosarcomas that developed in 5 of 13 guinea pigs following the subcutaneous injection of 3,4-benzpyrene dissolved in lard. Using the same carcinogen in a 5% concentration in cholesterol pellets, Shear [9d] obtained subcutaneous tumors in two guinea pigs 14 and 20 months after implantation. Warren and Gates [12] reported two fibrosarcomas and two liposarcomas in 53 guinea pigs, all of which survived more than 11 months following a single subcutaneous injection of 5 mg. of 3,4-benzpyrene in 0.5 cc. of glycerin. The average induction time for the tumors was 16 months.

In all the above studies the carcinogen was in contact only with mesenchymal tissues (subcutaneous fat or peritoneum) except for the work of Athias [9a] and Steiner,[10] in which the carcinogen was placed in the brain and the salivary gland, respectively. Therefore, it is not surprising that all of the tumors except the one reported by Athias were derived from mesenchymal cells.

10. Steiner, P. E.: Comparative Pathology of Induced Tumors of the Salivary Glands, Arch. Path. **34**:613-624 (Oct.) 1942.

11. Berenblum, I.: The Carcinogenic Action of 9,10-Dimethyl-1,2-Benzanthracene on the Skin and Subcutaneous Tissues of the Mouse, Rabbit, Rat, and Guinea Pig, J. Nat. Cancer Inst. **10**:167-174, 1949.

12. Warren, S., and Gates, O.: Spontaneous and Induced Tumors of the Guinea Pig, Cancer Res. **1**:65-68, 1941.

COMMENT

All of the tumors induced were one or another type of mesenchymal tumor. The tumors induced fell into a general spectrum of differentiation of mesenchymal cells from well-developed fibroblasts to such highly specialized cells as well-developed lipoblasts and osteoblasts. It is interesting to note that in some tumors the differtiation of lipoblasts was so high that the tumor could not be easily differentiated from a lipoma. Mixed-tumor types composed of liposarcoma, fibrosarcoma, and osteosarcoma were conspicuous, indicating the multipotential quality of the malignant cells. Variation in the rate of growth and the ability of the tumor to kill the animal was directly correlated with the differentiation of the tumor cells. As observed in Table 2, there were 41% fibrosarcomas, 31% liposarcomas, and 28% osteosarcomas.

The tumors reported in the literature, recorded in Table 3, that have been induced in guinea pigs by the carcinogenic stimulation of subcutaneous tissue were of three types of mesenchymal tumor (liposarcoma, fibrosarcoma, and osteosarcoma). This is what was observed in the study presented here. Totals of 56 fibrosarcomas, 26 liposarcomas, 15 osteosarcomas, and 2 unclassified sarcomas have been reported. Although there has not been sufficient work to warrant an unequivocal statement, it appears that methylcholanthrene is the most successful carcinogen for inducing subcutaneous tumors in guinea pigs.

The most striking thing to be observed in this group of tumors was the high percentage of liposarcomas. Liposarcomas are observed among induced subcutaneous tumors of mice but are rare. Haagensen and Krehbiel [9b] explained the high incidence of liposarcomas produced in guinea pigs by the fact that these animals have well developed subcutaneous fat pads and that the carcinogen acts directly on the fat cells to produce malignant transformation.

In cancer research the value of the guinea pig has been overlooked, owing to the difficulty of inducing tumors with carcinogenic substances. The size of this animal, as compared with the mouse, is desirable from the standpoint of handling, in addition to the ease with which the animal may be kept in the laboratory. Results of our study indicate that for the production of sarcomas of various types the guinea pig is a preferential animal, since tumors may be induced in a significant number of animals within a short period of time. Although our experiments did not show that the tumors were transplantable, the work of Shimkin and Mider [3] demonstrated that the tumors induced in guinea pigs were readily transplantable within inbred strains. Our failure to obtain successful transplantation of the tumors probably resulted from the use of a strain of animal that was not sufficiently homogeneous from the genetic standpoint. Transplantable liposarcomas, fibrosarcomas, and osteosarcomas can be produced in guinea pigs and made available for experimentation.

Recently interest in osteosarcoma has received impetus because of the affinity of radiogallium for osseous and dentigerous tissues. Because the malignant cells of these tissues store the metal similarly, opportunity is offered to deliver ionizing radiation selectively to malignant osseous tissues. The study of the problem of applying this knowledge to the treatment of osteosarcoma in man can be remarkably facilitated by having a conveniently transplantable osteosarcoma in a laboratory animal. The guinea pig is highly desirable because of the high incidence of osteosarcoma. This tumor has been induced in rabbits by the intravenous injection of

beryllium.[13] It appears, therefore, that the rabbit and the guinea pig may offer the best opportunity for experimental study of osteosarcoma.

Moreover, the high incidence of experimental liposarcoma affords an opportunity for studies of this type of tumor. Although this is a relatively rare type of sarcoma in man, the importance of having a suitable experimental animal for the study of this tumor should not be underestimated.

SUMMARY AND CONCLUSIONS

Malignant mesenchymal tumors that differentiated into liposarcoma, osteosarcoma, and fibrosarcoma have been induced in guinea pigs by the subcutaneous injection of methylcholanthrene. Tumors developed in 39 of 73 animals surviving after the first tumor was noted in the group. The tumors included 17 fibrosarcomas, 10 osteosarcomas, and 12 liposarcomas. Because of the comparative ease with which osteosarcomas and liposarcomas may be induced in guinea pigs, opportunity is offered to study these tumors in a desirable laboratory animal. The literature on tumors induced in guinea pigs by the carcinogenic polycyclic hydrocarbons is reviewed.

13. Dutra, F. R., and Largent, E. J.: Osteosarcoma Induced by Beryllium Oxide, Am. J. Path. **26**:197-209, 1950. Gardner, L. U., and Heslington, H. F.: Osteo-Sarcoma from Intravenous Beryllium Compounds in Rabbits, Federation Proc. **5**:221, 1946.

ATROPHY OF THE LEFT LOBE OF THE LIVER

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FROM THE accumulated experimental evidence of recent years, two basic concepts of hepatic disease have become increasingly apparent: (1) Identical injurious agents or diets may produce different responses in the liver that often are distributed unequally in its various portions and (2) several dissimilar types of injury can elicit nearly identical responses. One of the lesions occasionally observed is atrophy confined to or predominant in a single lobe of the liver or to focal areas in different lobes. Such foci of atrophy are exclusive of those obviously caused by mechanical compression, such as the "corset line" liver of human beings. In general, unilobar atrophy occurring in experimental animals has been observed to be cirrhotic or noncirrhotic, and to result from nutritional deficiencies,[1] disturbances of the portal blood supply or the free flow of bile, and organic and inorganic noxious agents. Unilateral lobar atrophy occurring in man has been infrequently reported in the literature [2] and then usually as an incidental curiosity encountered at necropsy. When present, its significance is often overlooked in the shadow of the underlying primary disease.

Necropsy records at the Mayo Clinic over a 30-year period reveal that severe atrophy confined to the left lobe of the liver occurs in portal cirrhosis, in postnecrotic cirrhosis, and in a variety of noncirrhotic diseases of the liver. Severe atrophy

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1. Gillman, J.: Brit. M. J. **1**:149, 1944. Gillman, J.; Gillman, T.; Mandelstam, J., and Gilbert, C.: Brit. J. Exper. Path. **26**:67, 1945.

2. (*a*) Frerichs, F. T.: A Clinical Treatise on Diseases of the Liver, translated by Charles Murchison, London, The New Sydenham Society, 1860, Vol. 1. (*b*) Froehlich, A., and Tverdy, G.: Acta chir. belg. Supp. 2, pp. 491-493, 1948. (*c*) Harrell, G. T., and McBryde, A.: Am. J. Dis. Child. **59**:1301, 1940. (*d*) Herringham, W. P.: St. Barth. Hosp. Rep. **41**:15, 1905. (*e*) Himsworth, H. P., and Glynn, L. E.: Proc. Roy. Soc. Med. **38**:101, 1944. (*f*) Kelsey, M. P., and Comfort, M. W.: Arch. Int. Med. **75**:175, 1945. (*g*) McIndoe, A. H., and Counseller, V. S.: Arch. Surg. **15**:589 1927. (*h*) Rokitansky, C.: A Manual of Pathologic Anatomy, translated by Edward Sieveking, London, The New Sydenham Society, 1849, Vol. 2, p. 125. (*i*) Rolleston, H., and McNee, J. W.: Diseases of the Liver, Gallbladder and Bile-Ducts, Ed. 3, London, Macmillan & Co., Ltd., 1929, p. 22.

confined to the right lobe was encountered only twice—once in association with "syphilitic" cirrhosis and once as a result of compression by a massive right adrenal tumor.

It was the purpose of this study to analyze the data in 32 cases of severe atrophy of the left lobe of noncirrhotic origin encountered in a 30-year period, with particular reference to the pathologic anatomy and pathogenesis of the condition.

MATERIAL AND METHODS

From the literature it is difficult to understand just what the limitations of normal are with regard to the left lobe of the liver, since many degrees of atrophy, particularly atrophy of the left posterior lateral tip, have been regarded simply as anatomic variations in this location. Histologic descriptions of these "normally" atrophic areas have received little attention. For this reason, it was considered necessary to examine a representative number of nonatrophic adult livers to establish in some detail the gross and microscopic anatomic characteristics of the left lobe, particularly in its distal half. This information was necessary as a basis for establishing minimal criteria for the presence of pathologic atrophy and for determining whether the latter differs in kind or in degree only.

The relationship of the left portal vein and the bile ducts was studied carefully in view of the cases reported by McIndoe and Counseller [2g] and Rolleston and McNee [21] in which atrophy of the left lobe was attributed to the pressure of a dilated left bile duct on the left branch of the portal vein. This relationship has not been emphasized in most standard texts of anatomy, though it has been well illustrated by Woerdeman [3] and Kopsch.[4]

Cases for this study were divided into two groups: control cases and cases in which there was severe atrophy of the left lobe.

Control Cases.—One hundred fifty adult livers with a minimal weight of 1,200 gm. were taken at random from necropsy cases encountered routinely; excluded were livers in which there was evidence of cirrhosis, biliary obstruction, gross distortion by metastatic tumors, and severe degrees of chronic passive congestion. The livers represented both sexes and all age groups from 20 to 80 years, inclusive. All livers were weighed and measured at the time of necropsy. Crude estimates of the relative sizes of the right and left lobes were made by using the time-honored line of division between the falciform ligament superiorly and the left sagittal fissure inferiorly. These estimates were based on the measurements of length, width and height of the respective lobes. It is realized that, owing to gross irregularities of the shape of the left lobe, these measurements represent at best only a rough approximation of actual relative sizes. In particular, the gross anatomic characteristics of the left posterolateral edge of the left lobe were noted in order to determine the extent of the appendix fibrosa hepatis and the degree of atrophy present.

At least one section was taken from the central portion of the hepatic parenchyma of each right lobe and each left lobe, where artifactitious distortion is minimal. In many cases, sections taken from the subcapsular regions at routine examination were also studied. In all cases, the number of perilobular portal triads per field at a magnification of 100 times was determined in sections taken from both right and left lobes and stained with hematoxylin and eosin. The average number of portal triads in three fields was computed in each instance.

In 25 cases the posterolateral tip of the left lobe with the corresponding triangular ligament was sectioned to study the structure of the fibrous appendix.

In 50 cases the porta hepatis was dissected to establish the anatomic relationship between the hepatic ducts and the portal vein and its branches.

Cases of Left Lobar Atrophy.—These cases were obtained from necropsy records of the Mayo Clinic over the 30-year period 1921 to 1950, inclusive. The only cases selected were those

3. Woerdeman, M. W.: Atlas of Human Anatomy: Descriptive and Regional, Philadelphia, The Blakiston Company, 1950, Vol. 2.

4. Kopsch, F.: Rauber's Lehrbuch der Anatomie des Menschen, Vol. 4, Eingeweide, Leipzig, Georg Thieme, 1914, p. 128.

in which either a gross anatomic diagnosis was made or a clear-cut description was given of atrophy which was confined to or predominant in the left lobe of the liver and which on further investigation was not associated with generalized cirrhosis. The criterion of cirrhosis employed was the presence of evidence of previous injury and the presence of both regenerative nodules and fibrosis. Thirty-two such examples were found, in all but one of which ample gross and microscopic material was available for study.

Three sections were taken routinely from each of the livers: one from the central portion of the right lobe, one from the left lobe, and, when possible, one from the junctional zone of the right and left lobes. The last-mentioned site included the area beneath the falciform ligament and across Cantlie's line. The number of perilobular portal triads per field at a magnification of 100 times was determined as in the control group. In cases in which there were gross anatomic changes in the portal vein or the bile ducts, additional sections were taken from the involved sites.

All tissues in this study were fixed in 10% formalin solution or Orth's fluid and were stained with hematoxylin and eosin. When indicated, the following special stains were used: Verhoeff's elastic stain with Van Gieson's counterstain, Gömöri's reticulum stain and Mallory-Heidenhain stain.

RESULTS

Control Cases

Study of the 150 livers used as controls confirmed previous descriptions of gross anatomic structure. The volume of the left lobe varied from one-fourth to one-seventh of the volume of the right lobe, the average for all age groups corresponding to the generally accepted figure of one-sixth. The distance from the falciform ligament to the left lateral border varied from 7 to 18 cm. Gross and microscopic changes consistent with minimal atrophy of the left lobe were confined to the distal third, most often within a few centimeters of the left triangular ligament. Variations of interest included five cases in which the left lobe was projected laterally and posteriorly in a long tongue-like process which was somewhat less firm in consistency than the remaining portion of the left lobe. The fibrous appendix of the left lobe was identified grossly in 90% of the cases. It appeared as a slip-like prolongation of the hepatic parenchyma varying in length from 1 to 6 cm. Histologic examination of the fibrous appendix revealed fibrosis, atrophy of the hepatic parenchyma, and thickening of the biliary and vascular channels. There were no other significant histologic differences between the right and the left lobe. The number of portal triads averaged three per field in both lobes. Flattening and distortion of the hepatic lobules were confined to those just beneath the capsule and adjacent to the fibrous appendix.

Examination of the 50 livers in which the porta hepatis was dissected disclosed a constant and important anatomic relationship. The right portal vein is virtually a continuation of the main stem, being short and large in caliber. The right hepatic duct runs roughly parallel to it. The left portal vein, on the other hand, is much longer and more slender and leaves the main stem at almost a right angle. It courses toward the left for 3 to 6 cm. before entering the left lobe of the liver, and is crossed superiorly by the left hepatic duct, so that probes inserted in both channels form an X. In eight cases the midportion of the left portal vein was covered inferiorly by the anterior tip of the caudate lobe.

Cases of Left Lobar Atrophy

The cases of atrophy of the left lobe represented all decades of life from the fourth to the ninth, inclusive, the youngest patient being 31 years old, the oldest 81. Nineteen of the patients were males, and 13 were females. As would be

expected, in those cases in which there was underlying disease of the biliary tract, females predominated over males 9 to 3.

Because the hepatic lesions were not apparent clinically, tests of hepatic function were not performed in many cases of this series. In the cases in which there were obstruction of the bile duct and portal thrombosis, tests of hepatic function, when performed, gave results that were compatible with the primary disease entity but apparently not specifically related to the left lobar atrophy. Moderate anemia was a common finding. Of 27 cases in which the hemoglobin was determined, the value was less than 12 gm. per 100 cc. in 15 cases and less than 9 gm. per 100 cc. in 7 cases.

Analysis of the principal clinical and gross anatomic features of the 32 cases in which the left lobe was atrophic allows the cases to be conveniently classified into five groups: (1) those in which there was obstruction of the portal vein or of its branches, (2) those in which there was evidence of present or previous obstruction of the bile ducts, (3) those in which there was evidence of biliary obstruction and compression of the left portal vein, (4) those in which severe malnutrition was the outstanding clinicopathologic feature, and (5) those in which there were miscellaneous features.

Group 1 (Six Cases).—In four cases, the clinical diagnosis was splenic anemia. Splenomegaly was of long duration in three cases (1 to 12 years) and of five weeks' duration in the fourth. Portal thrombosis occurred in the course of hypertensive toxemia of pregnancy in the fifth case.

In all cases of Group 1 there was occlusion of the portal system in whole or in part. In five cases there were thrombi in the tributaries and the main stem of the portal vein. In four of these there was evidence of both old and recent occlusion, and the spleens weighed more than 900 gm. In one of these cases there were recanalized thrombi in the hepatic veins and a hypertrophied caudate lobe.

In the sixth case the left branch of the portal vein was narrowed at its insertion into the left lobe to pinhole size by an encroaching metastatic tumor; no tumor nodules were present elsewhere in the left lobe. Cholelithiasis was present in three cases, with no evidence of obstruction of the bile duct.

Group 2 (Five Cases) and Group 3 (Seven Cases).—The cases associated with biliary obstruction represented the largest group in this series (12 cases, or nearly 40%). In several cases, disease of the biliary tract had occurred years before death. Present or past clinical jaundice was established in six cases. In four cases, surgical procedures had been performed on the gall bladder at some time in the past. In four cases there was no history of disease of the biliary tract, the diagnosis having been made at necropsy. It is interesting that in two cases exophthalmic goiter was present, disease of the biliary tract being of secondary importance and apparently not related to the immediate cause of death.

In the five cases of Group 2 the common bile duct was described as being dilated but there was no description of abnormalities of the portal vein. The diameter of the common bile duct varied from 1 to 3 cm. in three cases and was not recorded in two. The dilatation was due to choledocholithiasis (three cases) and to carcinoma of the common and left hepatic ducts (one case); in the remaining case the common bile duct was 2 cm. in diameter without demonstrable choledocholithiasis at necropsy.

The seven cases of Group 3 were characterized by obstruction and dilatation of the biliary tree with coincident narrowing of the lumen of the left branch of the portal vein. The dilatation resulted from choledocholithiasis (four cases), carcinoma of the head of the pancreas (one case), carcinoma of the common and the left hepatic duct (one case), and metastatic adenocarcinoma of the rectum (one case). In the last-mentioned case the metastatic growth compressed both the left hepatic duct and the left portal vein, with thrombosis of the vein and dilatation of the bile duct radicles distal to the point of compression. In all cases there was marked narrowing of the left branch of the portal vein, beginning just beyond the bifurcation of the main stem. These left branches of the portal vein were described as thickened, narrowed, or atrophic in the protocols. In all cases the main trunk and the right branch were normal. Despite the fact that the left portal vein and the left bile duct were adequately described in the protocols, their anatomic relation to each other was not recorded. Unfortunately, only two cases of obstruction of the common duct in this group could be satisfactorily restudied with respect to the bile-duct and portal-vein crossing. In the others there was insufficient gross material for study from this standpoint. In these two cases the obstruction was in the common duct, the left hepatic ducts being 1.5 and 1.8 cm. in diameter, respectively, at the point where they crossed the left portal vein. In both cases the left portal vein was compressed by the dilated left hepatic duct above and by the tip of the caudate lobe below. No thrombi were present in the left portal veins except in the case mentioned above.

Group 4 (Six Cases).—In all cases of this group, long-standing, severe malnutrition was of primary clinical concern. Of the six patients, one had carcinoma of the larynx, one had carcinoma of the lip with extensive surgical resection, and four had long-standing psychosis or mental deficiency that required institutionalization. One patient weighed 70 lb. (31.7 kg.) at the time of death. Three led what was described as a "vegetative existence" and had frequent vomiting. The patient with carcinoma of the larynx had a history of a six-month period of semistarvation and progressive severe cachexia.

With only two exceptions, the weights of the livers in this group indicated slight or moderate generalized atrophy. This was in keeping with decrease of body weight and generalized visceral atrophy, and did not correlate with old age. In each instance, however, the left lobe of the liver was strikingly more atrophic than the right. The bile ducts and the portal vein were normal.

Group 5 (Eight Cases).—This group consisted of eight cases of atrophy of the left lobe which, from the standpoint of available clinical and pathologic data, were unrelated. Histories were often inadequate concerning dietary habits, use of alcohol or drugs, and so forth, so that evaluation of such factors was not undertaken. In two cases a significant degree of cachexia was present, but these were not placed in Group 4, because emaciation was not a primary clinical consideration. Congestive heart failure, protracted vomiting and uremia, and roentgen therapy to the upper part of the abdomen were among the possible factors present in this group, but their relation to the occurrence of left lobar atrophy could not be clarified.

No anatomic factors were present to account for unilateral hepatic atrophy. The degrees of atrophy and the total weights of the livers did not differ significantly from those of other groups. The bile ducts and the portal vein were normal in each case.

Gross Features of Cases of Left Lobar Atrophy

Only six of the livers were suitable for measurement of the entire right and left lobes. In these the volume of the right lobe varied from 10 to 36 times that of the left. In one liver the left lobe measured only 2 by 2 by 1 cm. It was apparent from the protocols, photographs, and dimensions of the left lobe in relation to total weight of the liver in each case that the remaining livers had a volume ratio (right lobe to left lobe) of much more than 6 to 1 as observed in the controls. Characteristically, the capsules were diffusely and coarsely wrinkled, although in approxi-

Fig. 1.—Atrophy of the left lobe of the liver in a case of obstruction of the common bile duct, with compression of the left branch of the portal vein. Capsular wrinkling and prominent biliary and vascular channels are evident. Note that the junctional zone corresponds to Cantlie's line.

mately one-fourth of the cases the wrinkling was confined to the outer half of the lobe or was obscured by much fibrous thickening. Prominent tortuous vascular and biliary channels frequently lined the capsule (Fig. 1). The presence of capsular adhesions was unusual.

Other than the presence of occasional thrombi in the intrahepatic portal venules in the cases of Group 1 and bile staining and dilated biliary radicles in those of Groups 2 and 3 (Fig. 2), there were no distinctive features characteristic of any

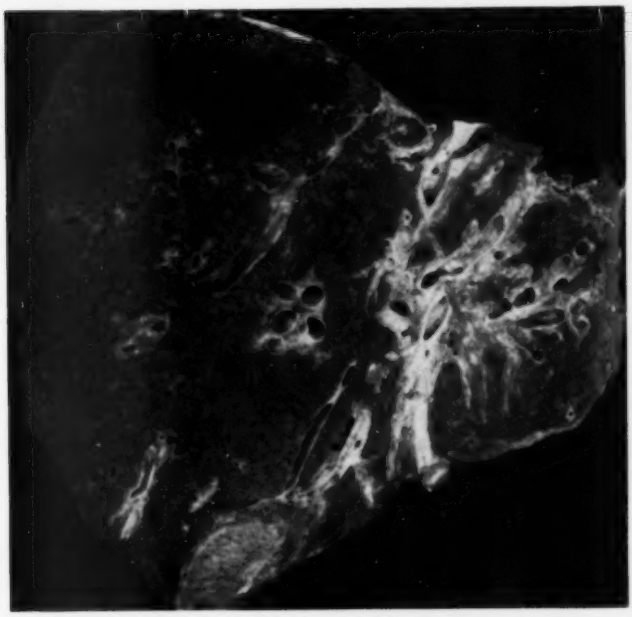

Fig. 2.—Transverse section of a liver in a case of obstruction of the bile duct due to carcinoma of the head of the pancreas. There is evident hydrohepatosis in the atrophic left lobe as compared with the right.

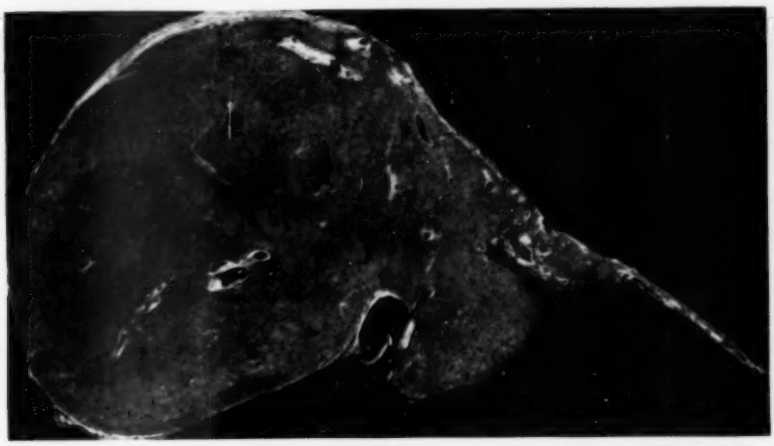

Fig. 3.—Spatulous atrophic left lobe of the liver in a case of choledocholithiasis.

one group on cut section. In the extremely small, spatulous left lobes the bulk of the parenchyma was replaced by fibrous tissue in which were crowded, prominent vascular and biliary channels. These resembled the fibrous appendices of the normal liver (Fig. 3). When the left lobes were larger, patches of atrophic parenchyma were visible, particularly in the central portions of the lobe and near the left margin. In 10 cases, fibrosis was apparent only in the subcapsular region of the lateral half of the lobe.

The entire fibrous appendix was included in the gross material in only 12 cases. It could not be definitely distinguished from the atrophic hepatic tissue adjacent to it, and in no case was there a definite zone of demarcation to set it off as a distinct structure.

Grossly, the junction between "normal" hepatic tissue and atrophic parenchyma was well defined in 13 cases, not discernible in 11 cases, and could not be evaluated from available material in 8 cases. When the border zone was sharp, it fell roughly on a line corresponding to a plane between the falciform ligament and the left sagittal fissure in eight cases and from 1 to 2 cm. to the right of this plane in five cases, the latter line corresponding to Cantlie's line in four instances.

In several cases the right lobe as judged by the total weight of the liver was interpreted as being hypertrophied.

Histologic Characteristics

The most important histologic features of these cases were thickening and coarse wrinkling of the capsule, increase in number of perilobular portal triads per field as compared with the right lobe (Fig. 4*a*), distortion and flattening of the lobules (Fig. 4*b*), absence of all but a few abortive regenerative nodules, vascular and bile-duct thickening, and variable degrees of fibrosis with increase of elastic fibrils in some cases. The extent of these histologic changes varied from case to case, and there were no constant patterns specific for any one of the five groups. In 12 cases the entire lobe was diffusely involved; in 5 the severe atrophic changes were confined to the lateral half of the lobe, and in 3 only a significant increase in number of portal triads per field accounted for the marked atrophy noted grossly. In the remaining cases the atrophic changes were irregularly distributed in the left lobe. Portions of the left lobe with atrophic lobules and an increased number of portal triads per field were encountered adjacent to portions in which there was little or no change. The greatest degree of atrophy was seen in the distal half of the lobe and in the subcapsular regions. However, in contrast to the control series, the subcapsular atrophy extended for a breadth of several lobules instead of the breadth of only the immediately subjacent row of lobules.

Fibrosis was extensive in 17 cases, moderate to slight in 9, and insignificant in amount in the remainder. It was considered extensive when the left lobe, in whole or in part, was replaced by fibrous tissue characterized by a richness in collagen and elastic fibrils that was out of all proportion to that seen in the controls (Fig. 5*a*). Fibrosis was considered moderate or slight when it involved only a portion of a lobe and was confined to the periportal region. Generally, the fibrosis was perilobular, extending inward from the capsule and portal canals. In some instances it obliterated most parenchymal structures completely. The reticular framework as delineated by the Gömöri reticulum stain was frequently collapsed

or obscured by thick reticulum strands that were scattered diffusely in the dense fibrous tissue (Fig. 5*b*).

Lobular distortion varied with the degree of fibrosis. Where fibrosis was minimal or absent, the lobules, instead of maintaining a polygonal structure with

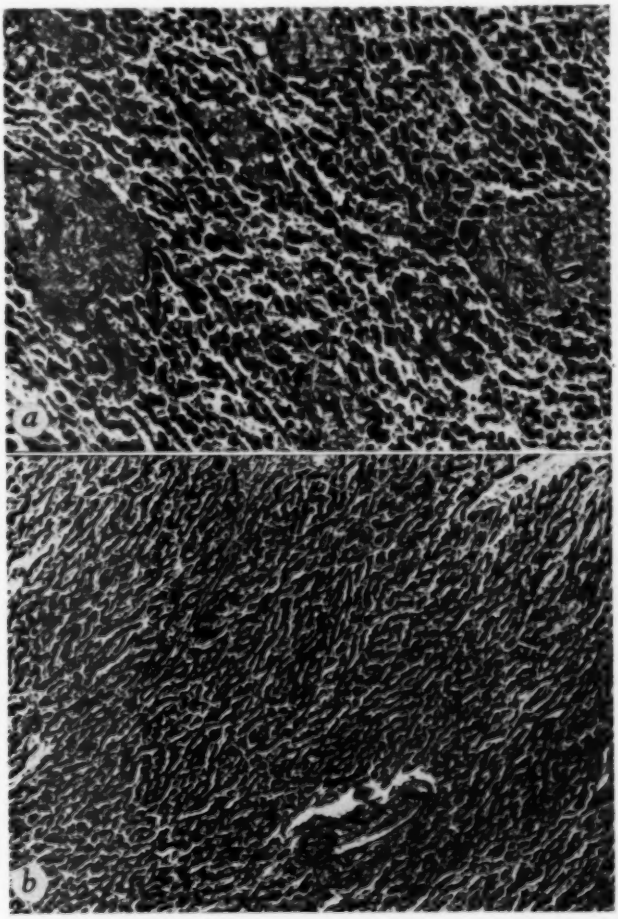

Fig. 4.—(*a*) Portion of a field from the left lobe showing increase in the number of portal triads. Seven of the 10 portal triads in the field are visible. The number of portal triads in the right lobe did not exceed 3 per field at the same magnification. (Hematoxylin and eosin; × 100.)

(*b*) Section from the middle of the left lobe. The stringing-out effect of the lobules, atrophy of the hepatic cords, and lack of regenerative nodules are to be noted. (Hematoxylin and eosin; × 65.)

cords of hepatic cells radiating outward from a central vein, tended to flatten so that columns of hepatic cells from several lobules coalesced to form long parallel

rows. The portal triads and central veins were closer together than normal but in their same relative positions. Some lobules, while retaining their polygonal shape, were smaller than normal, frequently having compressed sinusoids, atrophic hepatic cells, and prominent portal canals. Central veins were not displaced.

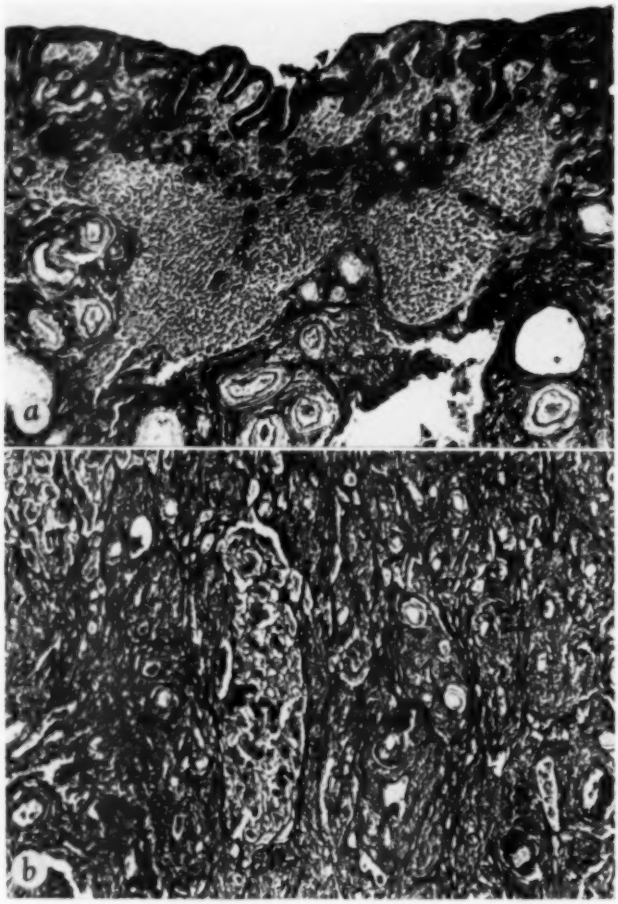

Fig. 5.—(*a*) Section of the left lobe showing coarse wrinkling and thickening of the capsule, concentration of portal triads, increase of elastic tissue, and thickening of bile ducts and vessels. There is absence of nodular regeneration. (Verhoeff's elastic stain counterstained with Van Gieson's stain; × 30.)

(*b*) Section from the left lobe showing collapsed reticular framework. (Gömöri's reticulum stain; × 100.)

Where fibrosis was extensive, only bizarre-shaped portions of lobules remained. These surviving fragments were crescentic, fusiform, or coarsely angulated but presented identifiable central veins in most instances. Their shape was determined

by the encroaching fibrous tissue and the relationship to bile ducts and large vessels. There were occasional small abortive regenerative nodules without central veins. In these cases the appearance was compatible with a diagnosis of portal cirrhosis although limited to the left lobe. Occasionally in the matrix of fibrous tissue, only isolated strings of hepatic cells remained. In general there was no correlation between the size of hepatic cells and the degree of lobar atrophy. Many individual cells had large hyperchromatic nuclei, but binucleated forms were seen infrequently. Mitotic figures were not encountered.

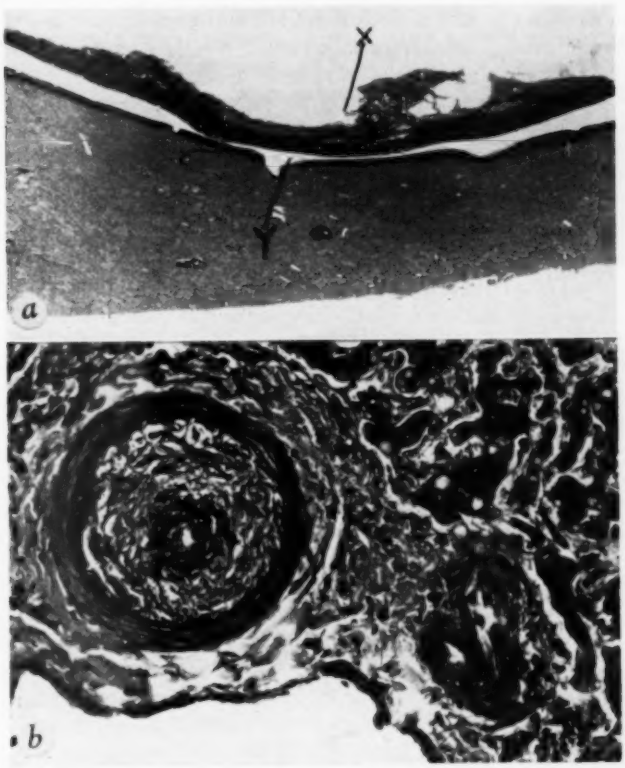

Fig. 6.—(*a*) Section of a conspicuously dilated left hepatic duct, *x*, where it crosses the left branch of the portal vein, *y*. There is compression of the vein with thickening of its wall in the center of the field. (Verhoeff's elastic stain counterstained with Van Gieson's stain; × 6.)

(*b*) Small artery in a portal triad of the left lobe showing severe luminal narrowing and intimal proliferation. There is a hyalinized arteriole at the lower right. (Hematoxylin and eosin; × 250.)

Necrosis of hepatic parenchyma was a prominent feature in only two cases of the group in which there was portal occlusion. The distribution of the necrosis was patchy in both cases. Minor degrees of centrolobular necrosis or fatty change were seen in those cases in which similar findings were presented in the right lobe.

Sections taken from the extrahepatic portion of the left portal vein at its junction with the left hepatic duct disclosed that the intima was thickened and that the vein was compressed by the dilated bile duct in two cases of Group 3 (Fig. 6*a*). The intrahepatic branches of the portal vein in the cases of Groups 2 and 3 had noncollapsed fibrous walls with luminal narrowing and distortion. In several cases the portal venules were compressed by the dilated intrahepatic biliary radicles.

Thrombi were seen in the intrahepatic branches of the portal vein in five cases, the thrombi in three of the cases being associated with portal thrombosis in the major extrahepatic tributaries. Both recent and ancient thrombi were encountered.

There was definite arterial and arteriolar sclerosis with luminal narrowing confined to or predominant in the left lobe in 19 cases (Fig. 6*b*). Intimal proliferation, intimal fibrosis, and medial hypertrophy were observed. In four cases the arteriolar lumina were conspicuously narrowed by hyaline thickening of the intima, which stained brick red with Mallory-Heidenhain stain. Fragmentation or increased thickness of the elastic lamina was commonly observed in the small arteries. In each case there was a striking difference in the appearance of the arteries and arterioles of the left lobe when they were compared with those of the right lobe.

In the cases of obstruction of the bile ducts there was a high incidence of severe lymphocytic and mononuclear cell infiltration in the portal canals of both lobes, and in one case there was suppurative cholangitis that was more striking in the left lobe. In other groups, inflammatory changes were not noteworthy.

The junctional zone of atrophic and nonatrophic hepatic tissue was more definite grossly than microscopically. Although a definite zone of demarcation could be seen in several cases, it was not sharply defined, extending over the thickness of several lobules and in an irregular fashion. In many instances no junctional zone could be found within a single section, this observation correlating with that in cases in which the atrophy was predominantly in the lateral half of the lobe.

COMMENT

This study suggests that several factors, perhaps interdependent, may be concerned in the production of atrophy of only the left lobe, among which the anatomic, nutritional, and probably toxic factors are most important.

Anatomic Factors.—There is little evidence to support the view that atrophy of the left lobe of the liver is developmental in origin. Complete absence of the left lobe is a rare anomaly. Merrill [5] reported the only case encountered in 19,000 necropsies performed at Johns Hopkins Hospital and found only one other case in a review of the literature. An atrophic left lobe occurring in a person less than 16 years of age has not been reported (the youngest patient in 10 cases of left lobar atrophy cited by Herringham [24] was 16 years old). Mayo Clinic records for a 30-year period contain no examples of complete absence of the left lobe; the youngest patient with left lobar atrophy was 31 years of age. Although it is not improbable that cases of congenital hypoplasia do occur, the rarity of left lobar atrophy in early life and the high incidence of associated disease of the portal vein

5. Merrill, G. G.: Arch. Path. **42**:232, 1946.

and bile ducts in this series make such an explanation unlikely for the occurrence of most atrophic left lobes.

The association of atrophy of the left lobe and obstruction of either a branch of the portal vein or a bile duct was evident in 56% of the cases in this series. It is well known experimentally that occlusion of a branch of the portal vein can produce atrophy of the portion of the liver supplied by that branch.[6] However, in five of the six cases of Group 1 of our series the portal obstruction was not localized to the left branch but involved also the main stem and tributaries of the portal vein.

The anatomic differences of the left and right portal branches are useful in explaining why the left lobe alone is atrophied when there is a thrombus in the main portal vein. The left portal vein is longer and of smaller caliber than the right branch and joins the main stem at an acute angle. It is probable that when the main stem of the portal vein is compromised by a thrombus, the loss of blood supply to the left lobe will be relatively greater than the loss to the right. Bazett [7] stated that if the mesenteric veins are clamped, pressure in the portal vein drops from a normal of 6 to 8 mm. of mercury to about 3.7 mm. Theoretically, this decrease in pressure of fluid with appreciable viscosity should affect more profoundly a tissue supplied by a long slender vessel than one supplied by a short, wide vessel. This concept is substantiated by the rarity of right lobar atrophy and the more frequent occurrence of left lobar atrophy in cases of partial portal occlusion.

Rous and Larimore [6b] showed that ligation of a branch of the common hepatic duct will produce atrophy of the corresponding lobe in the presence of intact portal circulation to the opposite lobe. Two cases illustrating this fact were encountered in this series: one, that of an intrinsic primary adenocarcinoma of the left hepatic duct, the other, that of an extrinsic metastatic tumor which invested the left hepatic duct. However, it is noteworthy that in both instances the left portal vein was also compromised. The portal circulation and the bile flow of the right lobe were normal in both cases. It remains to be explained why atrophy of the left lobe occurs selectively in obstruction of the common bile duct. It has been suggested by some investigators [8] that the atrophy is due to the pressure of the dilated left bile duct on the longer and more slender left portal vein. In our series, a thickened or "atrophic" left branch of the portal vein was described in five cases of dilatation of the common duct. In two of these a dilated left hepatic duct compressing the left portal vein was demonstrated grossly and microscopically. In each case the tip of the caudate lobe covered the site where the bile duct crosses the portal vein. This relationship could not be studied in the other cases, because the specimens remaining for study were unsatisfactory for this purpose. It is possible, however, that this mechanism obtains in the other cases of obstruction of the common duct with atrophy of the left lobe, since there is no other apparent reason for selective narrowing of the left portal vein. This change was not observed in the absence of obstruction of the biliary tract.

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6. (*a*) Mann, F. C., and Magath, T. B.: Am. J. Physiol. **59**:485, 1922. (*b*) Rous, P., and Larimore, L. D.: J. Exper. Med. **31**:609, 1920.

7. Bazett, H. C.: The Hepatic Circulation, in Bard, P.: Macleod's Physiology in Modern Medicine, ed. 8, St. Louis, C. V. Mosby Company, 1938, pp. 415-416.

8. McIndoe and Counseller.[2g] Rous and Larimore.[6b]

The close relationship of intrahepatic portal veins and dilated biliary channels is also important. Several examples of narrowed and thickened portal venules adjacent to dilated bile ductules were noted in cases in which there was obstruction of the common duct. Atrophy of the right lobe associated with obstruction of the common bile duct was not encountered in the absence of cirrhosis.

Nutritional and Toxic Factors.—The effect of "streamlining" of the portal blood upon the liver cannot be overlooked in the production of atrophy of the left lobe. Although its role may be significant in all groups of this study, it offers a most attractive explanation for the group characterized by malnutrition and cachexia. By the streamline effect is meant that in the portal vein the stream of blood from the superior mesenteric vein remains discrete from that of the splenic vein so that blood in the former flows predominantly to the right lobe of the liver, while blood in the latter flows predominantly to the left lobe. Since the superior mesenteric vein drains the small intestine and ascending colon, while the tributaries of the splenic vein arise in the gastrosplenic region and descending colon, it follows that the right lobe of the liver receives the bulk of the nutrient material from the absorptive portion of the intestinal tract.

There is strong experimental evidence to support the streamline concept. Copher and Dick [9] demonstrated that dye injected into the splenic vein flowed to the left lobes and did not mix appreciably in the portal vein with superior mesenteric venous blood. Likewise, dye injected into the superior mesenteric vein selectively stained the right lobes. Mann [10] injected gentian violent into the veins draining the pancreas in rats and found that only the left lobes of the liver were stained. Bollman and Mann [11] produced atrophic cirrhosis confined to the left lobes by injecting carbon tetrachloride into the spleen. A most convincing experiment was that of Hahn, Donald, and Grier.[12] Using mongrel dogs, they injected radioactive phosphorus (P^{32}) into the splenic, superior mesenteric and jugular veins, respectively, and found that in the first instance the radioactive phosphorus was concentrated in the left lobes, in the second, in the right lobes, and in the third there was no demonstrable difference.

Himsworth [13] has used the streamline phenomenon to explain the predilection of partial massive dietary necrosis for the left lobes of the liver in rats. Since the underlying cause of hepatic necrosis is dietary deficiency, the available nutrient material is probably absorbed from the small intestine into the superior mesenteric vein to flow to the right lobes. Under these circumstances the left lobes receive little, if any nutriment via the portal blood and thus are more susceptible than the right lobes to damage.

One is tempted to try to apply this concept to human material, but it is difficult to do so. The liver of man is anatomically different from that of many experimental animals, and controlled experimental conditions are obviously not feasible. Had-

9. Copher, G. H., and Dick, B. M.: Arch. Surg. **17**:408, 1928.

10. Mann, F. C.: J. A. M. A. **121**:720, 1943.

11. Bollman, J. L., and Mann, F. C.: Ann. Int. Med. **5**:699, 1931; cited by Mann.[10]

12. Hahn, P. F.; Donald, W. D., and Grier, R. C., Jr.: Am. J. Physiol. **143**:105, 1945.

13. Himsworth, H. P.: Lectures on the Liver and Its Diseases, Cambridge, Harvard University Press, 1947.

field and Garrod [14] attributed the rarity of unilateral atrophy in man to the anatomic fusion of the lobes of the liver. Nevertheless, the cases of Group 4 in our series, characterized by severe malnutrition, might by inference at least provide a human counterpart to experimental nutritional deficiencies in animals if the streamline phenomenon is used to explain selective or predominant atrophy of the left lobe. In all the cases of this series the role of toxic factors can be neither ignored nor accurately evaluated. The histories were frequently lacking in detailed information about dietary habits, use of alcohol and drugs, occupational exposures, and so forth.

In two cases the patients presented not only disease of the biliary tract but also exophthalmic goiter. The association of diffuse toxic goiter and disease of the liver, particularly cirrhosis, is well known and has been extensively studied by several investigators.[15] Weller [15a] and Rössle [15b] regarded the hepatic lesions as toxic in nature. Rössle expressed the opinion that the lesions were most prominent in the subcapsular area owing to the diffusion of toxic products to this region. In regard to our two cases it may be suggested that in exophthalmic goiter the effect of accelerated metabolism, principally catabolic, would be most apparent in the left lobe since, according to the streamline theory, it receives the least nourishment.

That the left lobe of the human liver is more severely involved than the right by some forms of injury has been shown by Lucké [16] in infectious hepatitis, and by Stewart [17] and Turnbull [18] in trinitrotoluene poisoning.

Thus it may well be that although anatomic and nutritional factors are of prime importance in producing atrophy of the left lobe, toxic factors must be considered as possible contributory causes in all cases.

SUMMARY AND CONCLUSIONS

On the basis of a study of the pathologic features of 32 cases of severe atrophy of the left lobe of the liver encountered at the Mayo Clinic during a 30-year period it is concluded that the characteristic gross features of this lesion are obvious decrease in size of the left lobe with wrinkling and thickening of the capsule, fibrosis, and prominent biliary and vascular markings. The atrophic changes may be diffuse or patchy. They may involve the entire left lobe but are most severe in the distal half and in the subcapsular region.

Histologically, the most constant change is an increase in the number of perilobular portal triads per microscopic field of the left as compared with the right lobe. Other frequently observed findings are a thickly rugated capsule, flattening and distortion of the lobules, fragmentation of parenchymal elements, absence of all but abortive regenerative nodules, dense fibrosis, mural thickening of biliary and vascular channels, and arterial and arteriolar sclerosis with luminal narrowing.

In 75% of the cases in this series severe atrophy of the left lobe was associated with obstruction of the portal vein or of its left branch, obstruction of the biliary

14. Hadfield, G., and Garrod, L. P.: Recent Advances in Pathology, ed. 5, Philadelphia, The Blakiston Company, 1947, p. 247.

15. (*a*) Weller, C. V.: Tr. A. Am. Physicians **45**:71, 1930. (*b*) Rössle, R.: Arch. path. Anat. **291**:1, 1933. (*c*) Beaver, D. C., and Pemberton, J. deJ.: Ann. Int. Med. **7**:687, 1933. (*d*) Moschcowitz, E.: Arch. Int. Med. **78**:497, 1946.

16. Lucké, B.: Am. J. Path. **20**:471, 1944; **22**:867, 1946.

17. Stewart, M. J.: Proc. Roy. Soc. Med. **10**(Pt. I):10, 1917.

18. Turnbull, H. M.: Proc. Roy. Soc. Med. **10**(Pt. I):47, 1917

tract, partial obstruction of the lumen of the left branch of the portal vein associated with distention of the left hepatic bile duct, or malnutrition and cachexia.

The anatomic, nutritional, and toxic factors are discussed in relation to the pathogenesis of atrophy of the left lobe in the light of known experimental facts. Bile stasis and anatomic peculiarities of the left portal vein are considered to be of significance. The streamline phenomenon of the portal blood offers an explanation for left lobar atrophy in the group of cases in which this is associated with malnutrition and cachexia. It also offers an explanation for the occasional occurrence of left lobar atrophy when the damage to the liver has been diffuse. In these cases it is suggested that in the right lobe regeneration is more nearly complete than in the left lobe because nutrition is better in the former.

Unilateral atrophy confined to the left lobe of the liver comprises an entity that is morphologically distinct from cirrhosis.

RENAL SIDEROSIS IN RABBITS FOLLOWING INJECTIONS OF HEMOGLOBIN AND ORGANIC IRON

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IT HAS been proposed that ferric iron liberated in the body on degradation of oxyhemoglobin may exert a toxic effect on the renal epithelium similar to that of heavy metals.[1] This hypothesis is a modification of an earlier proposal which suggested that renal tubular degeneration or necrosis might be due to hematin released during hemoglobinemia.[2] Since compounds containing ionizable iron are toxic,[3] the above theories should be evaluated by experimental evidence. We believe that the understanding of the etiology of hemoglobinuric nephrosis would be advanced by an appreciation of the role which iron plays in this syndrome. To attain this goal, experiments were performed in which alterations of renal function and microscopic changes in the renal tubules were compared with the presence of demonstrable iron in the kidneys.

METHOD

Histochemical studies for iron were done on kidneys from three groups of animals: 50 rabbits with hemoglobinuric nephrosis, 14 animals that received injections of organic iron, and 10 controls. Thirty-four rabbits with hemoglobinuric nephrosis had been without food or water for five days. The oxyhemoglobin was administered intravenously during the last three days of this period. The significance of reductions of extracellular fluid volume[4] and aciduria[5] relative to the production of hemoglobinuric nephrosis have been reported elsewhere. Sixteen other rabbits in the first group, maintained on either alkaline (rabbit pellet[6]) or acid (ground oats) diets, received intravenous injections of modified hemoglobin solutions in one day. Solutions of buffered hemin, ferrogluconate and ferric citrate were parenterally administered to

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1. Hampton, J. K., Jr., and Mayerson, H. S.: Am. J. Physiol. **160**:1, 1950.

2. Anderson, W. A. D.; Morrison, D. B., and Williams, E. F., Jr.: Pathologic Changes Following Injections of Ferrihemate (Hematin) in Dogs, Arch. Path. **33**:589 (May) 1942.

3. (*a*) Goetsch, A. T.; Moore, C. V., and Minnich, V.: Blood **1**:129, 1946. (*b*) Meyer, H., and Williams, F.: Arch. exper. Path. u. Pharmakol. **13**:70, 1880. (*c*) Starkenstein, E., and Weden, H.: Ibid. **150**:354, 1930.

4. Lalich, J. J.: J. Exper. Med. **87**:157, 1948.

5. Lalich, J. J., and Schwartz, S. I.: J. Exper. Med. **92**:11, 1950.

6. The pellets used were "master-mix" rabbit pellets made by the McMillen Feed Mills, Division of Central Soya Company, Inc., Fort Wayne, Ind.

another group as indicated in Table 4. The control rabbits were fed an acid diet of oats, followed by five days without food and water. Two other control rabbits were fed pellets and then deprived of food and water for three days. In order that the fluid intake might be comparable to that of rabbits receiving hemoglobin, the control animals were given an equivalent fluid volume of 5% intravenous dextrose. Plasma nonprotein-nitrogen (NPN) levels were ascertained by Conway's microdiffusion method[7] before and after the injections of hemoglobin. The plasma nonprotein-nitrogen changes were usually observed on the fifth day after the initial hemoglobin injection. The oxyhemoglobin solutions were prepared as described previously.[4] Rabbits which did not die after injections were killed as specified in the tables. The organs were examined, and samples of tissue were fixed in 10% formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin to demonstrate the degree of pigment cast accumulation as well as the extent of tubular injury. Adjacent sections were stained for ferric ion by Gomori's or Perls's method.[8] Kidneys from each group were also studied for the presence of ferrocomplexes.[9] Because of the gross and microscopic changes of experimental hemoglobinuric nephrosis have been described previously,[10] the significant renal changes will be listed only in the tables. Oxyhemoglobin degradation products or pigment casts accumulate

TABLE 1.—*Tubular Alteration in Rabbits Subjected to Withdrawal of Food and Water and Supported by Intravenous Injections of 5% Glucose*

Rabbit	Diet*	Highest Recorded Plasma NPN, Mg. per 100 Cc.	Autopsy, Days	Combined Kidney Weights, Gm.	Kidney (Microscopic Sections—H and E‡) Eosin Casts	Tubular Necrosis	Gomori's Test	Perls's Test
1	A	62	8	11.6	+	—	—	—
2	A	80	6	11.2	—	—	—	—
3	A	90	1†	13.8	—	+	+	—
4	A	56	6	15.6	+	—	—	—
5	A	58	3†	11.5	+	—	+	—
6	A	144	3†	11.5	+	+	+	—
7	A	60	6	9.4	+	—	—	—
8	A	58	6	13.6	+	—	—	—
9	B	..	3	....	—	—	..	—
10	B	..	3	....	+	—	..	—

* Diet A = ground oats plus 1% NaCl. Diet B = rabbit pellets.
† The rabbit was found dead.
‡ H and E = stained with hematoxylin and eosin.

principally in the distal convoluted tubules. The pigment casts are readily distinguished from proteinaceous eosin casts in hematoxylin-eosin sections by their yellow or orange color.[11]

Although quantitation of pigment casts is admittedly arbitrary, some approximation appears desirable, since it permits a numerical expression of pigment accumulation. The cast counts which are included in the tables represent the total number of casts in 10 low-power fields selected at random in the renal cortex.

RESULTS

Control Group.—The urine was rendered acid (pH 5.0-6.0) by feeding oats for two to three weeks prior to the withdrawal of food and water in eight control rabbits. Multiple injections of 5% intravenous dextrose were given on the last three days.

7. Conway, E. J.: Micro-Diffusion Analysis and Volumetric Error, London, Crosby-Lockwood & Son, 1939, Chap. 12.

8. Perls, M.: Arch. path. Anat. **39**:42, 1867. Gomori, G.: Am. J. Path. **12**:655, 1936.

9. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948, p. 125.

10. Lalich.[4] Lalich and Schwartz.[5]

11. Lalich, J. J.: Am. J. Path. **25**:187, 1949.

Feeding these rabbits oat diets and depriving them of food and water produced tubular degeneration or necrosis and eosin casts. These procedures also caused an elevation in plasma nonprotein nitrogen. That the limit of safety had been exceeded is evident, since three of the eight rabbits died. Gomori's test revealed an exceedingly faint bluish discoloration characteristic for iron within the epithelial cells of the proximal convoluted tubules in the three rabbits which died. In these three instances 12 or more hours had elapsed between death and formalin fixation of the tissues. Postmortem autolysis could very easily have caused these positive results.[12] It is interesting to note that sections from these three rabbits were negative to Perls's test.

Two other rabbits of the control series were fed pellets and deprived of food and water for three days before autopsy. Perls's test was negative in both cases.

TABLE 2.—*Renal Alterations Following Intravenous Hemoglobin Injections in Rabbits Deprived of Food and Water for Five days*

Rabbit	Diet*	Highest Recorded Plasma NPN, Mg. per 100 Cc.	Autopsy, Days	Combined Kidney Weight, Gm.	Total Pigment Casts in 10 Low-Power Fields	Gomori's Test	Perls's Test
11	A	300	5†	19.2	238‡	—	..
12	A	91	2†	10.5	11	+	+
13	A	96	1†	14.1	17	+	+
14	A	275	8†	22.6	193‡	—	..
15	A	48	13	15.8	0	+	+
16	A	198	9†	19.4	167	+	+
17	A	225	10†	19.2	281	+	+
18	A	42	13	14.3	0	+	+
19	A	135	8†	23.6	329	+	+
20	A	45	13	15.1	2	..	+
21	B	440	3†	20.1	318‡	+	+
22	B	360	6†	21.5	299	+	+
23	B	278	7†	27.8	414‡	+	+
24	B	94	10	13.9	2	+	+
25	B	59	10	15.8	14	+	+
26	B	316	5†	22.7	281‡	+	+

* Diet A = ground oats with 1% NaCl. Diet B = 3 parts oats, 1 part alfalfa with 1% NaCl.
† The rabbit was found dead.
‡ Rabbit had focal tubular necrosis.

Experimental Groups.—Observations on 16 rabbits fed acid diets and deprived of food and water for five days are described in Table 2. On the last three days a total of 1.8 gm. of hemoglobin per kilogram of body weight was given intravenously. The urine was acid (pH 5.0-6.0) before the withdrawal of food and water.

Eleven of the 16 rabbits died of hemoglobinuric nephrosis from 1 to 10 days after the initial hemoglobin injection. There is adequate agreement in the elevations of plasma nonprotein nitrogen, pigment-cast counts, and increases of kidney weight to indicate that they died in renal failure. In sections from 14 of the 16 animals ferric iron was demonstrated by either Gomori's or Perls's test. In the five rabbits with focal tubular necrosis it was not possible to demonstrate greater accumulations of free iron in the necrotic areas than elsewhere. It is of interest that in 2 of the 16 rabbits[13] with focal tubular necrosis there was no

12. Brown, W. H.: J. Exper. Med. **12**:623, 1910.
13. (*a*) O'Donnell, W. M.: Am. J. Path. **26**:899, 1950. (*b*) Lillie.[9]

demonstrable iron. Positive reactions, when encountered, were similar to those described by O'Donnell.[13a] The diffuse or granular bluish cytoplasmic alteration was present in the epithelial cells of the proximal convoluted tubules. Ferric iron was observed to a lower degree in the lumina and rarely in the pigment casts. Accumulations of free iron occurred in the proximal convoluted tubules, whereas focal tubular necrosis was usually observed in Henle's loops or the distal convoluted tubules.

Perls's test was positive for iron on the kidneys of 12 of 18 rabbits not included in Table 2. A negative reaction in the case of one rabbit which was killed 40 days after hemoglobin injections is understandable. It is not so apparent, however, why another rabbit which had many pigment casts after an interval of 14 days should have a negative Perls test. Four rabbits dying within 24 hours after hemoglobin injections did not exhibit ferric iron. The kidney sections from these

TABLE 3.—*Renal Lesions in Rabbits Maintained on Alkaline or Acid Diets Receiving Intravenous Injections of Modified Hemoglobin Solutions*

Rabbit	Diet*	Pigment Injected, Gm./Kg.†	Highest Recorded Plasma NPN, Mg. per 100 Cc.	Autopsy, Days	Combined Kidney Weight Gm.	Total Pigment Casts in 10 Low-Power Fields	Gomori's Test	
							Intracellular	Intraluminal
45	A	1.0 M	..	7	12.6	2	+	—
46	A	1.0 M	28	8	20.0	44	+	+
47	A	1.0 M	22	8	22.4	23	—	—
48	A	1.0 M	48	7	20.6	0	—	—
49	B	1.0 M	64	11	26.2	229	—	—
50	B	1.0 M	34	9	20.8	333	+	+
51	B	1.0 M	82	8	23.9	168	+	+
52	B	1.0 M	64	8	23.2	160	—	—
53	A	1.0 R	28	7	14.4	3	—	—
54	A	1.0 R	22	8	23.3	25	+	—
55	A	1.0 R	25	8	16.3	0	—	—
56	B	1.0 R	28	11	12.2	0	+	+
57	B	1.0 R	28	8	14.5	58	+	—
58	B	1.0 R	28	8	12.5	46	+	+
59	B	1.0 R	40	8	16.5	13	+	+

* Diet A = rabbit pellets. Diet B = 12 parts, soybean meal; 55 parts, ground oats; 33 parts, cerelose—to which was added a 2% salt mixture of the following: NH_4Cl, 15%; NaCl, 41%; KCl, 6%; KH_2PO_4, 38%.
† M = methemoglobin. R = acidified and reneutralized hemoglobin.

animals were subjected to hydrogen peroxide oxidation [14] and then stained according to Perls's technic. Following hydrogen peroxide oxidation the pigment casts revealed hemosiderin by Perls's test in three of four instances, whereas the epithelial cells remained negative.

In the second group the effect of modified hemoglobin solutions was evaluated in 15 rabbits.

They were fed alkaline or acid diets and were not subjected to food or water deprivation. Methemoglobin was prepared by mixing 2 ml. of 1% sodium nitrite with 5 ml. of a solution of oxyhemoglobin (9-13 gm. per 100 cc.) before it was injected into 10 animals. The oxyhemoglobin was acidified to a pH of 5.0 with 0.1-normal hydrochloric acid and then neutralized to 7.4 with 0.1-normal sodium hydroxide before it was injected into the remaining six rabbits. Acidification denatures some of the hemoglobin; hence it is necessary to filter the solution prior to injection. A total of 1 gm./kg. of modified hemoglobin solution was injected intravenously in two to four doses within a period of one day.

14. Brown, W. H.: J. Exper. Med. **8**:290, 1911

No rabbit in this group died and only 1 of 15 had a nonprotein-nitrogen value in excess of 75 mg. per 100 cc. of plasma. The greatest number of pigment casts were observed in rabbits which were maintained on acid diets and given methemoglobin parenterally. It is of interest to observe that the kidneys were also heaviest in this group. Gomori's test was positive in 9 of 15 instances. Ferric iron was encountered more frequently within the epithelial cells. In four of the six cases in which negative reactions were encountered, there were few or no pigment casts. In these four cases the negative reactions could be accounted for on the basis that either hemoglobin had been eliminated from the kidneys or the pigment had been deposited in the reticuloendothelial system during the interval (7 to 11 days) following injection. In the two other cases, however, the many pigment casts present in the renal tubules indicated that elimination was delayed. The finding of

TABLE 4.—*Renal Lesions in Rabbits Following Injections of Various Organic Iron Salts*

Rabbit	Diet*	Organic Iron Salt, %	Injections and Route†	Total Quantity of Iron Injected, Mg./Kg.	Autopsy, Days	Kidney (Microscopic Sections—H and E§)	Gomori's Test
60	B	0.3 hematin	I. V. 2 days (5×)	3.0	6	Eosin casts; renal infarcts	+
61	A	0.3 hematin	I. V. 2 days (5×)	2.8	2‡	Thrombi; renal infarcts	+
62	A	0.3 hematin	I. V. 1 day (1×)	1.8	12 hr.‡	Renal infarcts	—
63	A	0.2 hematin	I. P. 1 day (2×)	3.9	9	Eosin casts	—
64	A	0.2 hematin	I. V. 2 days (4×)	1.8	10	Normal	—
65	A	0.2 hematin	I. P. 1 day (2×)	3.8	9	Normal	—
66	A	0.5 ferro-gluconate	I. V. 1 day (6×)	18.9	5 hr.‡	Focal necrosis	+
67	A	0.5 ferro-gluconate	I. P. 1 day (2×)	13.6	24 hr.‡	Normal	—
68	A	0.5 ferro-gluconate	I. V. 2 days (3×)	9.1	12	Normal	+
69	A	0.5 ferro-gluconate	I. V. 6 days (4×)	11.3	4	Eosin casts	—
70	A	0.5 ferro-gluconate	I. P. 1 day (2×)	4.8	10 hr.	Normal	+
71	A	0.5 ferric citrate	I. V. 1 day (3×)	7.1	24 hr.‡	Eosin casts	—
72	B	0.5 ferric citrate	I. V. 2 days (3×)	9.7	8	Normal	—
73	A	0.5 ferric citrate	I. V. 1 day (3×)	8.0	2‡	Normal	—

* Diet A = rabbit pellets. Diet B = ground oats plus 1% NaCl.
† I. P. 2 days (3×) = three intraperitoneal injections in a two-day period.
‡ The animal was found dead.
§ H and E = stained with hematoxylin and eosin.

many pigment casts in kidneys in which ferric iron is not demonstrable suggests that pigment casts and ferric iron may occur independently.

In the last group of 14 rabbits a comparison is made between the toxic effects of organic iron salts and the retention of demonstrable iron in the renal tubules.

The hemin [15] was prepared by dissolving 200 or 300 mg. of the powder in 15 ml. of 0.1-normal sodium hydroxide. Once the powder was dissolved, 50 ml. of water was added and the solution buffered to a pH of 7.0-7.3 with 0.1-normal hydrochloric acid. The volume was then diluted to 100 ml. before injection. Five hundred milligrams of ferric citrate was dissolved in 70 to 80 ml. of hot water. The solution was cooled to room temperature, buffered to a pH of 7.0-7.3 with 0.1-normal sodium hydroxide, and then diluted to 100 ml. The ferrogluconate [16] solution

15. The hemin was purchased from Eastman Kodak Company, Rochester, N. Y.

16. The ferrogluconate was supplied by Eli Lilly & Company Research Laboratories, Indianapolis.

was prepared in a similar manner. The number of injections, the route of administration, and the total quantity of iron injected, calculated in milligrams per kilogram (mg./kg.), are shown in Table 4.

Two of six rabbits given hemin died after the injections. Kidneys from three were considered normal, whereas infarcts were encountered in the kidneys of three others. A study of kidneys from five of these hemin rabbits revealed ferric iron in two. Two of five rabbits given the largest ferrogluconate injections died within one day. Eosin casts were observed in kidneys of two rabbits; however, focal tubular necrosis occurred in only one of these. The kidneys of the remaining three rabbits were normal. Iron was demonstrated in one rabbit which died and two rabbits which survived. Two of the three rabbits given ferric citrate died within two days. The kidney sections were not unusual except for the presence of moderate numbers of eosin casts. Iron was not demonstrable in any of the kidneys from these three rabbits. Of the three organic salts studied, hemin was the most toxic. It produced death and renal injury at much lower iron concentrations than the other two organic salts. Iron was demonstrated in kidney sections from 5 of 13 rabbits given organic iron salts. The iron was localized generally in the proximal convoluted tubules. However, in no way did the accumulations of demonstrable iron approach in magnitude those observed in the hemoglobinuric nephrosis series. This is noteworthy since three to four times as much iron was injected into the last group.

Turnbull's blue reaction for ferrous ion [9] was done on renal sections which were positive for the ferric ion. As a control, animals negative for the ferric ion were chosen at random from all the series, and their renal tissues were analyzed for ferrous ion. No ferrous ion was demonstrable in the tissues from any of these rabbits.

COMMENT

It has been possible to confirm the findings of Rather in rats [17] by demonstating ferric iron in the epithelial cells of the proximal convoluted tubules of rabbits after intravenous injections of hemoglobin. Pigment casts invariably stained negatively for iron, which is in agreement with Lucké's observations.[18] When renal sections from rabbits which died within two days after hemoglobin injections were oxidized with hydrogen peroxide, ferric iron was released in a few casts. The greatest concentrations of iron occurred in the epithelial cells of the proximal tubules, to a lower degree in the lumina, and rarely in pigment casts. A few of the animals with hemoglobinuric nephrosis, as well as others, which died after injections of organic iron had no demonstrable iron in the renal tubules. It was not possible to establish any relationship between accumulation of iron in the tubules and alteration of renal function. Since iron accumulates in the proximal tubules and focal tubular necrosis is encountered in Henle's loops or the distal tubules, we believe that tubular necrosis can be attributed to other factors. Focal tubular necrosis developed in only one of the rabbits which received excessive injections of organic iron.

A solution of potassium ferrocyanide will form Prussian blue granules whenever ferric ions are present in an acid medium.[19] To differentiate ferritin from hemo-

17. Rather, L. J.: J. Exper. Med. **87**:163, 1948.

18. Lucké, B.: Mil. Surgeon **99**:371, 1946.

19. Granick, S.: Chem. Rev. **38**:379, 1946.

siderin or colloidal ferroalbumin complexes, chemical determinations in addition to Prussian blue reaction are necessary. A negative ferric iron test does not exclude ferric iron. This element may be bound as in methemoglobin or denatured globin hemochrome or bound as ferro-iron in hemoglobin or denatured globin hemochrome.[20] Some of the pigment excreted in urine is present as hemoglobin, methemoglobin, or hemochromogen. Since pigment casts are in part composed of the above compounds, a negative Prussian blue reaction is understandable. The presence of blue dye due to ferro-iron has been excluded, since Turnbull's reaction was always negative.

The fact that iron was not demonstrable in the tubules of some rabbits after injection of organic iron salts implies several possibilities: The injected iron was bound, it may have been stored elsewhere and did not reach the kidneys, or it was excreted in the urine. Urinary excretion of more than minimal quantities of parenterally injected free iron does not occur.[21] Starkenstein has shown that the toxicity of iron salts and the extent to which they disappear from the blood and are deposited in the liver and the spleen are appreciably modified by the chemical structure of the iron compound.[3c] His observations, as well as our own, show that the toxicity of iron salts is not solely a matter of iron concentration per se. The negative Prussian blue reaction in kidney sections from a majority of rabbits given organic iron salts suggests that the iron was deposited elsewhere.

To resolve the matter of whether iron is nephrotoxic, it seems that several variables must be considered. First, to exert physiologic or toxic effects, the iron must be free or ionizable.[3c] Second, the quantity of iron released must attain toxic levels. Finally, the chemical structure in which the ionizable iron exists is also important.[3c] It is reasonable to assume that the role of iron toxicity in hemoglobinuric nephrosis should be evaluated with respect to the quantity of iron which may be released following a mismatched blood transfusion. Intravascular hemolysis may exert a lethal effect when the concentration of hemoglobin approaches 1 gm. per kilogram. A severe hemolysis of this degree, calculated from the molecular weight of hemoglobin, represents 40 mg./kg. of hemin [22] or 3.4 mg./kg. of iron.[23] Since 30 to 40% of the plasma hemoglobin may be excreted in the urine,[24] the concentration of iron remaining in the body capable of exerting a nephrotoxic effect can be approximated. If all of the iron retained in the body ionizes and exerts a nephrotoxic effect, it must do so at concentrations below 3.4 mg./kg. of iron. Of the organic salts injected, only hemin caused death and produced renal infarcts at dose levels approximating 3.4 mg./kg. of iron. Similarly, it was not possible to produce focal tubular necrosis or anuria by parenteral injections of hemin, ferrogluconate, or ferric citrate at iron concentrations equal to 3.4 mg./kg. We believe the above observations are especially significant, since negative results were encountered even after the concentration of 3.4 mg./kg. of iron was exceeded by appreciable amounts.

20. Lemberg, R., and Legge, J. W.: Hematin Compounds and Bile Pigments, New York, Interscience Publishers, Inc., 1949, p. 223.

21. McCance, R. A., and Widdowson, E. M.: Lancet **115**:680, 1937; J. Physiol. **94**:148, 1938.

22. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, ed. 3, Baltimore, Williams & Wilkins Company, p. 72.

23. Morrison, D. B., and Hisey, A.: J. Biol. Chem. **109**:233, 1935.

24. Hamilton, P. B.; Hiller, A., and Van Slyke, D. D.: J. Exper. Med. **86**:477, 1947.

SUMMARY

Iron in the renal tubules may be used in association with other factors to establish a diagnosis of hemoglobinuric nephrosis as suggested by O'Donnell.[13a] Our observations do not indicate that demonstrable iron per se is responsible for the physiologic or pathologic changes which may be observed in hemoglobinuric nephrosis. The above experiments do not exclude the possibility that ferric iron is exerting some influence on the tubules; however, we believe this role to be of minor importance in the etiology of hemoglobinuric nephrosis. It should be stressed that when ionizable iron exerts a toxic effect, the toxicity is not due solely to the iron concentration but is appreciably modified by the accompanying anion. The observation of less iron in the tubules following injections of organic iron salts suggests that these salts are stored at other sites more readily than is hemoglobin.

MOBILIZATION OF MUCOPROTEIN BY PARATHYROID EXTRACT

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OVER 25 years ago Collip [1] proved that the tetanic state of the parathyroidectomized animal is associated with a lowered level of serum calcium. He also showed that this condition can be controlled by the administration of an acid extract of the parathyroid gland which contains the parathyroid hormone in some form. In normal animals treated with the hormone by injection the serum calcium levels became elevated and large amounts of phosphate were excreted. Continued administration of this substance induced various toxic symptoms, such as polyuria, anorexia, and intestinal hemorrhage, and finally led to death. Although it is clear from the work of Collip and many others that the parathyroid gland regulates the metabolism of calcium and of inorganic phosphorus, the manner in which this is achieved is not well understood.

One group of investigators [2] holds that the hormone acts directly on the bone, causing it to release calcium, which elevates the serum calcium level. The high level of calcium in the serum is then thought to depress the concentration of phosphorus in the serum. Another group of workers [3] have believed that the primary site of action of the parathyroid hormone is the kidney, where the threshold for phosphate is lowered. This is said to depress the level of phosphate in the serum. The ability of the serum to dissolve calcium is then presumably increased, and calcium is withdrawn from the bone. The bone lesion is thus considered a secondary phenomenon, contributing to homeostasis.

On the basis of an approach somewhat different from that of earlier investigations, the view has been advanced that the changes in bone following the

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1. Collip, J. B.: Extraction of a Parathyroid Hormone Which Will Prevent or Control Parathyroid Tetany and Which Regulates Level of Blood Calcium, J. Biol. Chem. **63**:395, 1925; The Parathyroid Glands, Harvey Lectures, 1925-1926.

2. (*a*) Jaffe, H. L., and Bodansky, A.: Experimental Fibrous Osteodystrophy (Osteitis Fibrosa) in Hyperparathyroid Dogs, J. Exper. Med. **52**:669, 1930. (*b*) McLean, F. C., and Bloom, W.: Calcification and Ossification: Mobilization of Bone Salt by Parathyroid Extract, Arch. Path. **32**:315, 1941. (*c*) Selye, H.: Mechanism of Parathyroid Hormone Action, ibid. **24**:625, 1942.

3. Tweedy, W. R.; Chilcote, M. E., and Patras, M. C.: Distribution, Retention, and Excretion of Radiophosphorus Following Thyroparathyroidectomy, or Bilateral Nephrectomy, and Administration of Parathyroid Extract, J. Biol. Chem. **168**:597, 1947. Albright, E., and Reifenstein Jr., E. C.: Parathyroid Glands and Metabolic Bone Disease, Baltimore, Williams & Wilkins Company, 1948.

administration of parathyroid hormone may be a direct result of the hormone action. The central point of interest is considered to lie in the nature of the organic matrix of bone and the labile state of the ground substance of connective tissues.

In a fundamental study, Gersh and Catchpole [4] proposed that the ground substance of connective tissue is organized as a highly polymerized carbohydrate-protein complex. This could be broken down to smaller residues, possibly by mucolytic enzymes secreted by connective tissue cells. It was emphasized that the state of organization of ground substance must be regarded as being highly plastic. As a corollary to this it was considered likely that the serum mucoprotein might represent a soluble derivative of the ground substance. Experimental evidence for this view was later given by Catchpole.[5]

The ground substance of bone is regarded here as a highly polymerized glycoprotein. Though relatively insoluble and chemically inert under some conditions, it is highly labile in certain physiologic and pathologic states. Our purpose in this investigation has been to show that the hormone of the parathyroid gland is implicated in maintaining the labile state of the organic matrix.

This has been demonstrated by histochemical studies of the ground substance of the bone of rats which had received injections of parathyroid extract, coupled with a chemical study of some carbohydrate-protein complexes of serum and bone. The histochemical reaction of the altered bone was interpreted as evidence of depolymerization of the ground substance. At the same time, serum analysis showed mucoproteins to be present in increased amount, sometimes reaching five times that of the normal animal. When massive doses of hormone were used, the lumina of the kidney tubules were filled with carbohydrate-containing material presumably derived from the mucoprotein of the serum.

These observations led us to the tentative generalization that the parathyroid hormone acts in part on the connective tissue of bone, possibly through stimulating the liberation of enzymes which can depolymerize the ground substance of bone and of cartilage. As mucoprotein residues of the ground substance, derived from bone and epiphysial cartilage diffuse into the blood, the serum is altered. With the release of the ground substance of the bone, bone salts are simultaneously liberated into the blood, whence they reach the kidney.

MATERIALS AND METHODS

Young rats of the Sprague-Dawley strain, weighing 90 to 270 gm., were used. They were maintained on the routine laboratory diet, consisting of a commercial chow [6] and water, fed ad libitum. The animals were given varying amounts of parathyroid extract [7] in single and divided doses by injection. There were five groups of animals in the experiment (Tables 2 to 5):

Group 1 (control series). Some of the rats received no injections; others received 0.9% sodium chloride or dilute bovine gamma globulin.

Group 2 (small doses). These rats were given from 10 to 30 units of parathyroid extract by injection and were killed after 19 hours.

4. Gersh, I., and Catchpole, H. R.: The Organization of Ground Substance and Basement Membrane and Its Significance in Tissue Injuries, Disease and Growth, Am. J. Anat. **85**:457, 1950.

5. Catchpole, H. R.: Serum and Tissue Glycoproteins in Mice Bearing Transplantable Tumors, Proc. Soc. Exper. Biol. & Med. **75**:221, 1950.

6. This was purina® fox chow, made by the Ralston Purina Company, St. Louis.

7. Eli Lilly & Company contributed a generous supply of their parathyroid extract.

Group 3 (intermediate doses). From 100 to 500 units of parathyroid extract was injected, and the animals were killed after 24 hours.

Group 4 (massive doses). From 800 to 1,600 units of parathyroid extract was injected, and the animals were killed after 24 to 72 hours.

Group 5 (300 units of parathyroid extract). The rats were killed at 1 to 48 hours.

Blood was withdrawn from the right side of the heart under ether anesthesia for determination of the serum mucoproteins. The animals were killed and tissues were removed directly for histochemical and chemical studies.

Histochemical Studies on Bone and Kidney.—Slices of the knee joint and of the kidneys were frozen in 2-methyl-butane (isopentane) chilled in liquid nitrogen to about —150 C. and dried in a vacuum at about —30 C. The blocks were infiltrated with paraffin at 60-62 C. in a vacuum. The tissues were then cut at thicknesses varying from 2 to 16 μ, without preliminary decalcification. The sections were deparaffinized in xylene or petroleum ether. The glycoproteins and other carbohydrate-containing components of these tissues were visualized with the aid of the periodic acid-leucofuchsin method.[8] In most cases this was done only after the tissue proteins had undergone preliminary denaturation in absolute alcohol for 24 hours so as to render reactive

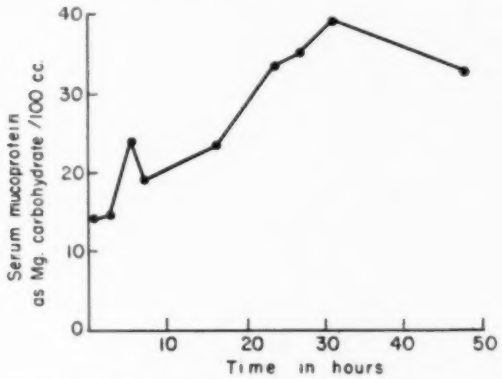

Fig. 1.—The change in serum mucoprotein level with time following administration of 300 units of parathyroid extract.

proteins insoluble. Other sections were treated with buffers and enzymes before staining in order to define the carbohydrate complexes qualitatively.

The carbohydrate-containing material noted in the kidney tubules was also studied with the ultraviolet microscope. The preparations for this purpose were made from the kidneys of five animals receiving over 1,000 units of parathyroid extract by injection during three days. Thin sections (2 μ) which previously had been denatured in formalin-ethanol 1:1 (v/v) for 24 hours at room temperature were mounted in glycerin on quartz slides under quartz cover glasses. The transmission of light at a series of wave lengths between 2480A. and 3650A. was recorded and plotted as a "modified extinction," i. e., $\log \frac{I_0}{I}$.

Localization of inorganic phosphate-carbonate was done by the silver nitrate method described by Gersh.[9]

8. McManus, J. F. A.: The Histological Demonstration of Mucin After Periodic Acid, Nature **158**:202, 1946. Hotchkiss, R. D.: A Microchemical Reaction Resulting in the Staining of Polysaccharide Structures in Fixed Tissue Preparations, Arch. Biochem. **16**:131, 1948.

9. Gersh, I.: Improved Histochemical Methods for Chloride, Phosphate-Carbonate, and Potassium Applied to Skeletal Muscle, Anat. Rec. **70**:311, 1938.

Analytical Methods.—Mucoprotein determinations were made on the serum by the method of Winzler and associates.[10] The proteins were removed with perchloric acid, leaving the mucoproteins, which were precipitated from the filtrate with phosphotungstic acid. The mucoprotein was then analyzed for carbohydrate and tyrosine, and the carbohydrate-tyrosine ratio was calculated. A soluble mucoprotein component of the bones was extracted by the method for connective tissue described by Catchpole.[5] The bones about the knee joint, including approxi-

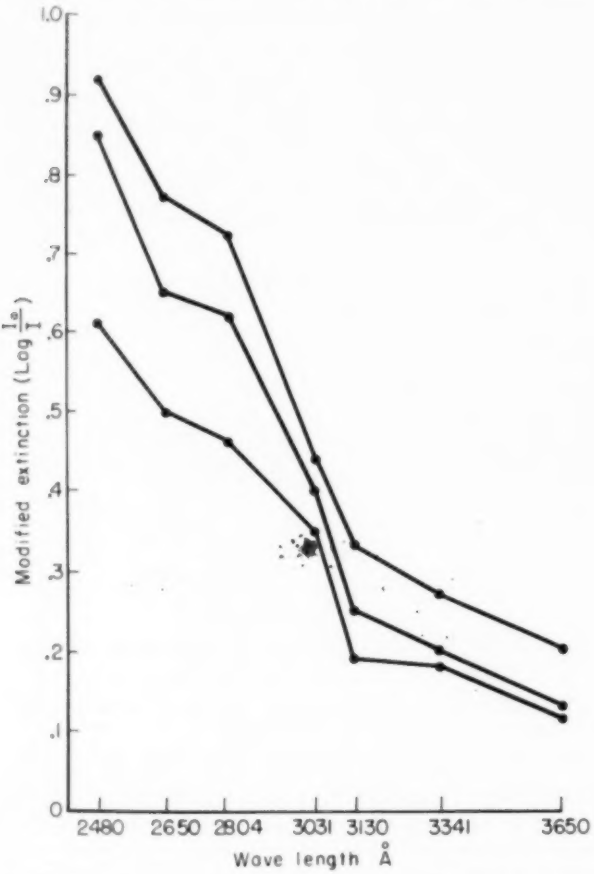

Fig. 2.—Ultraviolet extinction curves of tubular casts plotted from data obtained from three rats receiving three injections totaling 1,200 units of parathyroid extract in 72 hours.

mately one-third of each limb, were dissected. Muscle and tendon were removed as much as possible, and the bones were frozen-dried. The dried bones were then further freed of all soft tissues and ground with 5 ml. of methanol-chloroform 1:1 (v/v) and extracted at 37 C. in 20 ml. of methanol-chloroform for three hours to remove fats. The supernatant fluid was

10. Winzler, R. J.; Devor, A. W.; Mehl, J. W., and Smyth, I. M.: Studies on the Mucoproteins of Human Plasma I, J. Clin. Invest. **27**:617, 1948.

poured off and replaced with an equal volume of acetone. In one hour the acetone was decanted and replaced with ethanol. The ethanol was removed after approximately 16 hours, and the tissues were washed with acetone and dried in air over phosphorus oxide (P_2O_5) to constant weight. The denatured, defatted residue was then ground with exactly 2ml. of M/15 phosphate buffer at pH 7.0 and extracted in the cold for one hour.

OBSERVATIONS

Histochemical Observations on Bone.—The sections through the knee joints of control rats show sites of osteogenic and osteolytic activity typical of normal young animals. The zone of primary spongiosa, which contains cartilage and cartilage spicules enveloped by bone (Fig. 3*A*), stains purplish red with the periodic acid-leucofuchsin reagents. It is a characteristic of young bone that its stainability is heightened in comparison with that of the older diaphysial bone. The latter stains less intensely, having a pink tinge, while the shaft bone of older animals barely reacts with the reagent. Cobb[11] has proposed that this variation in the degree of staining or reactivity of the bone is a function of its degree of polymerization—the greater the degree of polymerization the less the intensity of the staining reaction, since fewer reactive groups are thought to be available.

This characteristic pattern was altered in rats that received injections of parathyroid extract. The findings agree essentially with those of Heller.[12] Changes were readily observed in animals receiving at least 500 units of hormone after 24 hours and became exaggerated at higher doses. The area of primary spongiosa disappeared or was largely reduced, in which case fragments of ground substance and some fibrous remnants of bone and cartilage remained (Fig. 3*B* to *D*). Many of the macrophages contained red-staining granules and larger aggregates which were not affected by amylase digestion and which appeared to be phagocytosed remnants of bone or cartilage (Fig. 3*E*). Many of these cells also contained deposits of insoluble phosphate-carbonate (Fig. 3*F*). In some sections stained for both carbohydrate and phosphate-carbonate it appeared as though the two groups of substances were often associated with each other in the intracellular aggregates. Diaphysial bone, which ordinarily is but weakly reactive, stained intensely red. Multinucleated cells, probably osteoclasts, were identified in some areas, but this was not a consistent finding. It appeared that the bone changes were not dependent upon the presence of these multinucleated cells.

Microscopic spaces in the connective tissues surrounding bone spicules, produced by ice crystals, were a characteristic feature of many of the preparations from the parathyroid-extract-treated animals; they were not prominent in control animals. It is probable that this artifact of freezing-drying fixation reflects the highly fluid character of ground substance resulting from the depolymerization of bone glycoprotein.

Determination of Alcohol-Insoluble, Water-Soluble Mucoprotein of Bone.—A group of 24 animals comprising 12 parathyroid-extract-treated and 12 saline-treated

11. Cobb, J.: The Morphological Distribution of Glycogen and Glycoproteins in the Cells and Extra Cellular Materials of Growing Bones, M.S. Thesis, University of Illinois, 1948.

12. Heller, M.: Ground Substance, Bone Salts, and Cellular Activity in Bone Formation and Destruction, Ph.D. Thesis, University of Chicago, 1951. Heller, M.; McLean, F. C., and Bloom, W.: Cellular Transformations in Bones, Am. J. Anat. **87**:314, 1950.

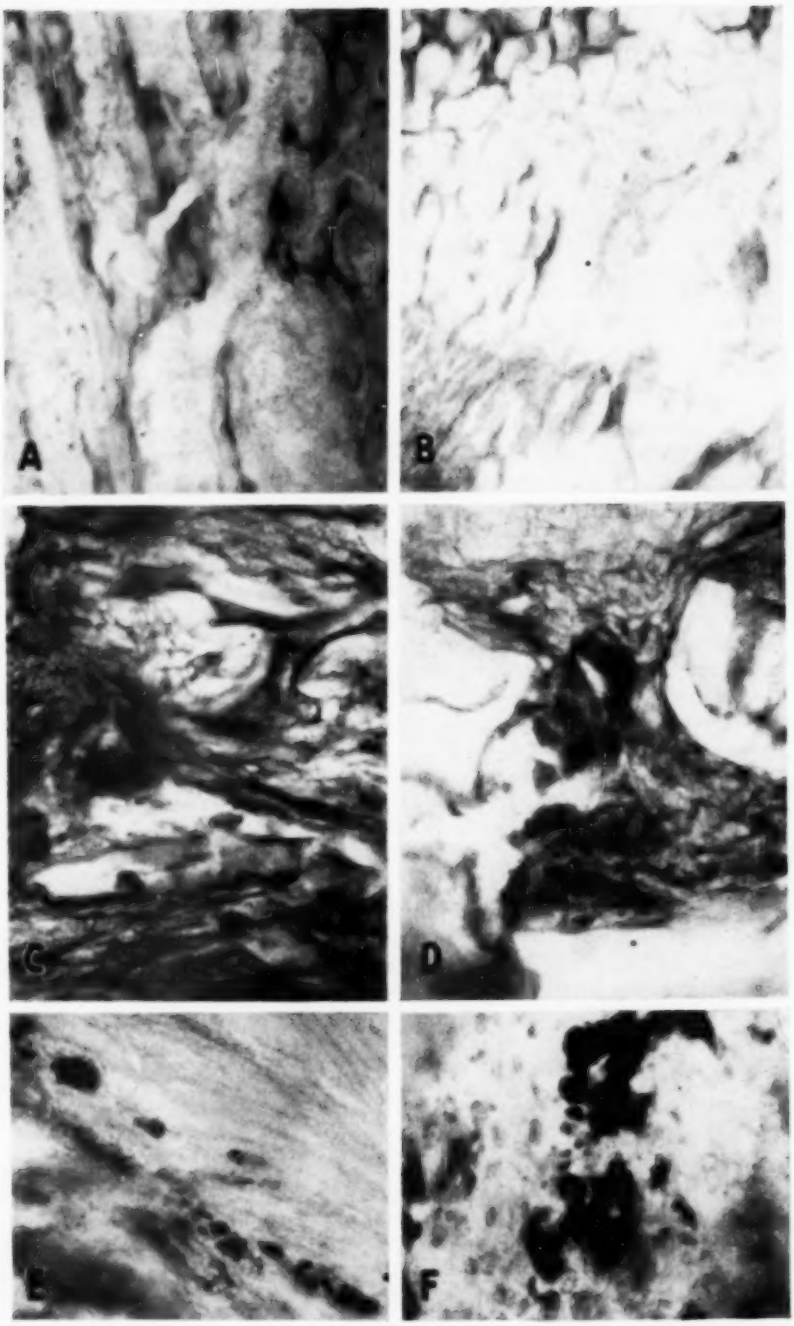

Figure 3

(See legend on opposite page)

controls (12 cc. of 0.15 M NaCl) were included in this experiment. Massive doses of parathyroid extract, 1,100 to 1,200 units, were given during a three-day period. Such large doses had been shown to cause extensive changes in bones which were studied by histochemical methods. The alcohol-insoluble, water-soluble mucoprotein was extracted from the dry, defatted bones with phosphate buffer (M/15, pH 7.0) in the manner previously described, and analytically determined (Table 1).

In the control group this alcohol-insoluble, water-soluble carbohydrate-containing component was present in amounts varying from 48.2 to 132.0 mg. per 100 gm. of dry, defatted bone. The mean value was 92.5 mg. per 100 gm. In the series of rats receiving parathyroid extract the bones contained from 49.6 to 195.2 mg. per

TABLE 1.—*Alcohol-Insoluble, Water-Soluble Mucoprotein of Bone Expressed as Carbohydrate (Mannose-Galactose)*

	Controls (12 Cc. of 0.15 M NaCl) *	Experimental Rats (Parathyroid Extract—1,100-1,200 Units) *
Individual values, mg. carbohydrate/100 gm. of dry defatted bone	48.2	49.6
	56.3	79.8
	57.0	98.5
	62.0	105.2
	74.0	113.2
	84.0	121.5
	85.4	138.0
	114.8	159.5
	124.0	166.5
	126.0	183.0
	132.0	190.9
	145.8	195.2
Mean	92.5 ± 9.8 †	133.3 ± 13.3 †
t	2.45	

When $t = 2.508$, $P = 0.02$; therefore when $t = 2.45$, $0.05 > P > 0.02$

* The animals received three divided doses by injection 72, 42, and 18 hours before being killed.
† Standard error of the mean.

100 gm. The mean value was 133.3 mg. per 100 gm. The difference between the two groups is shown to be statistically significant, with P approaching a value of 0.02. The results are interpreted as showing a significantly increased amount of

EXPLANATION OF FIGURE 3

All the tissues represented here were fixed by freezing-drying. The preparations shown were stained for carbohydrate-containing substances with the periodic acid-leucofuchsin reagents after ethanol denaturation.

A, spicules of young bone from the primary spongiosa of a control rat. Some of the spicules contain dark cartilage cores; × 290.

B, area from primary spongiosa of a rat receiving three injections totaling 1,150 units of parathyroid extract in 48 hours. The animal was killed 25 hours after the last injection. There are only a few remnants of bone and cartilage; × 290.

C to *F*, dissolution of bone following three injections totaling 1,150 units of parathyroid extract in 72 hours. The animal was killed 24 hours after the last injection. *C* shows the spicules and surrounding connective tissue deeply stained. Some of the fibers appear to be continuous with the depolymerized bone matrix; × 650. In *D* the large dark-staining aggregates are disintegrating pieces of bone spicules; × 650. In *E* the macrophages in the connective tissue adjacent to dissolving bone contain glycoprotein granules in their cytoplasm; × 1,200. The preparation seen in *F* was also treated to demonstrate inorganic phosphate-carbonate. The black masses are phosphate-carbonate (presumably calcium salts) deposits. Some of these are intracellular and are associated with phagocytosed glycoprotein; × 615.

water-soluble, alcohol-insoluble mucoprotein in the bones of the parathyroid-extract-treated animals.

Serum Mucoprotein Level.—The serum mucoprotein level in 16 rats that received no injections (Group 1) was 15.9±0.8 mg.[13] per 100 cc. expressed as carbohydrate and 4.1±0.2 mg. per 100 cc. expressed as tyrosine. No appreciable changes were observed in several animals receiving sodium chloride and bovine gamma globulin (Table 2).

The animals in Groups 2 to 4 received doses of parathyroid extract[14] which were roughly graded between low and massive. The relationship between the total dose of hormone and the serum mucoprotein level was obscured by variations in timing of postinjection blood sampling and uncertainty regarding the time of maximal response. In a general way it seemed as though the mucoprotein level was related to the dose (Tables 3 to 5).

Small Doses: The carbohydrate levels ranged from 23.3 to 25.7 mg. per 100 cc.; the tyrosine values, from 5.4 to 6.3 mg. per 100 cc.

TABLE 2.—*Control Animals*

Animals	Treatment	Wt., Gm.	Serum Mucoprotein as Carbohydrate, Mg./100 Cc.	Serum Mucoprotein as Tyrosine, Mg./100 Cc.	Carb./Tyr.
16	Untreated	100-200	15.9 ± 0.8*	4.1 ± 0.2*	3.9 ± 0.3*
	10 cc. of 0.1% bovine gamma globulin (24 hr.)	151	20.9	6.6	3.2
3	0.1 cc. of 0.1% bovine gamma globulin (18 hr.) †	191 202	18.9	4.5	4.2
1	8 cc. of 0.9% NaCl (18 hr.)	...	18.4	5.1	3.6

* Standard error of the mean.
† Mean values for carbohydrate and tyrosine are given.

Intermediate Doses: The range for carbohydrate was from 27.6 mg. per 100 cc. (C) to 40.0. These corresponded to 100-unit and 500-unit doses, respectively.

Massive Doses: Even higher values were recorded in this group. One rat receiving 1,180 units in a 48-hour period had a carbohydrate level of 73 mg. per 100 cc. with the tyrosine at 21.5 mg. per 100 cc., almost a fivefold rise of the mucoprotein level as compared with the normal. Some of the animals in this group died.

This rise of the serum mucoprotein level was followed in a series of animals given a single injection of 300 units and then killed at varying intervals during an over-all period of 48 hours (Fig. 1). A peak in the carbohydrate level of 39 mg. per 100 cc. was reached at about 31 hours. Even at 48 hours the level remained high, 33 mg. per 100 cc. In most animals receiving parathyroid extract the carbohydrate-tyrosine ratio showed only small deviations from the mean of 3.9 observed in the normal and control series.

Renal Changes.—When more than 1,000 units of hormone was administered during periods ranging from 43 to 96 hours, anuria developed in some rats, which became very ill or died. At autopsy the kidneys were dotted with yellowish-gray specks and the cut sections had intensified markings in both cortex and medulla.

13. Standard error of the mean.

14. Analysis of the parathyroid extract showed it to contain virtually no mucoprotein.

On microscopic examination it was noted that there were cystic areas in the cortex and that the tubules were often dilated. Many of the tubules were plugged with homogeneous polysaccharide-containing masses or aggregates of closely packed

TABLE 3.—*Group 2. Animals Receiving One Dose of 10-30 Units of Parathyroid Extract and Killed in Nineteen Hours*

Wt., Gm.	Dose, Units	Serum Mucoprotein as Carbohydrate, Mg./100 Cc.	Serum Mucoprotein as Tyrosine, Mg./100 Cc.	Carb./ Tyr.
128	10	25.7	6.3	4.1
136	20	24.0	5.4	4.4
133	30	23.3	5.7	4.1

TABLE 4.—*Group 3. Animals Receiving 100-500 Units of Parathyroid Extract and Killed Twenty-Four Hours After Initial Injection*

Wt., Gm.	Dose, Units	Serum Mucoprotein as Carbohydrate, Mg./100 Cc.	Serum Mucoprotein as Tyrosine, Mg./100 Cc.	Carb./ Tyr.
226	100	27.6	7.5	3.7
237	200	29.8	8.3	3.6
241	200	28.6	7.1	4.0
125	200	39.9	7.7	5.2
90	300	33.8	7.6	4.4
125	500 (2 inj.) *	40.5	...	...

* Animal was killed 17½ hours after the second injection.

TABLE 5.—*Group 4. Animals Receiving a Total Dose of 800-1,600 Units of Parathyroid Extract in Multiple Injections and Killed in Twenty-Four to Ninety-Six Hours After the Initial Injection*

Wt., Gm.	Dose, Units	Injection Intervals, Hr.*	Duration of Experiment, Hr.	Serum Mucoprotein as Carbohydrate, Mg./100 Cc.	Serum Mucoprotein as Tyrosine, Mg./100 Cc.	Carb./ Tyr.
112	800 (2 inj.)	24, 16½	24	37.9	9.6	3.9
178	980 (2 inj.)	43½, 40	43½	49.0	6.8	7.2
114	1,000 (3 inj.)	24, 19½, 3	24	35.5	7.9	4.5
272	1,000 (2 inj.)	43½, 37	43½	58.5	15.5	3.8
190	1,150 (3 inj.)	48, 31, 24	48	64.8	18.7	3.5
178	1,180 (3 inj.)	48, 31, 24	48	73.0	21.5	3.4
192	1,200 (3 inj.)	48, 31, 24	48	67.0	17.8	3.8
156	1,200 (3 inj.)	48, 31, 24	48	67.7	15.9	4.3
...	1,300 (4 inj.)	96, 72, 48, 24	96	70.8	15.8	4.5
...	1,300 (4 inj.)	96, 72, 48, 24	96	48.0	10.3	4.7
143	1,600 (4 inj.)	46, 43, 22, 2	48	45.5	....	...

* The hours entered here refer to intervals elapsing between the several injections and the time at which the animal was killed.

granules (Fig. 4*A* and *D*). In some instances the cells of the tubules, particularly those of the proximal convoluted tubules, contained large glycoprotein granules (Fig. 4*B*). Deposits of phosphate-carbonate were demonstrated in the lumina of the tubules and in their lining cells (Fig. 4*C* and *D*). A few of these sections were subsequently stained for polysaccharide. In some areas glycoprotein casts contained deposits of phosphate-carbonate (Fig. 4*D*). The carbohydrate-containing material was shown to be soluble in dilute buffers and electrolytes over the pH range 1-11.

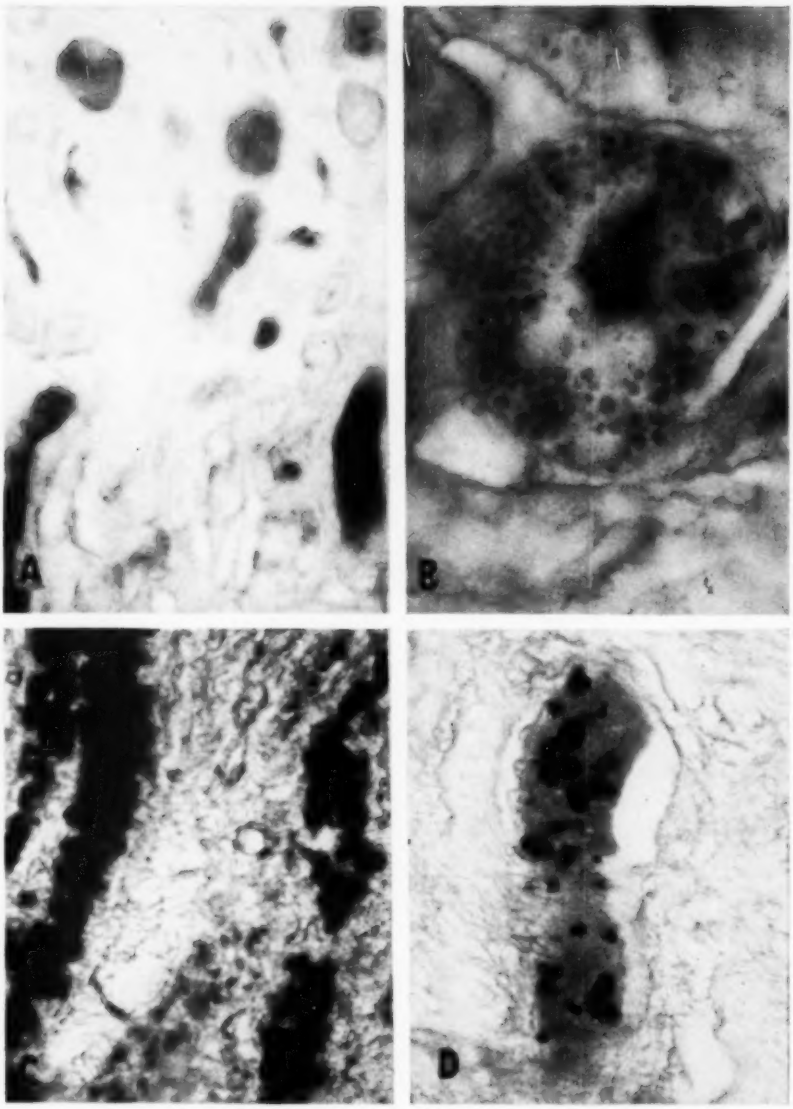

Fig. 4.—Changes in the kidney following three injections totaling 1,200 units of parathyroid extract in 48 hours. The rat was killed 24 hours after the last injection. All the tissues represented here were fixed by freezing-drying. All the preparations shown except that in *C* were stained for carbohydrate-containing substances with the periodic acid-leucofuchsin reagents after ethanol denaturation.

A, kidney tubules containing glycoprotein plugs; × 625.

B, large intracellular glycoprotein granules in cross section of a proximal convoluted tubule; × 1,200.

C, section through kidney tubules prepared to visualize phosphate-carbonate only; × 650.

D, preparation treated to demonstrate inorganic phosphate-carbonate. The glycoprotein cast in a dilated kidney tubule contains phosphate-carbonate deposits; × 650.

Absolute ethanol, saturated with mercury bichloride, and ethanol-formalin 1:1 (v/v) were used in an attempt to denature the material. Only after use of ethanol-formalin was it possible to avoid solution of this substance in phosphate buffer (M/15, pH 7). Treating these denatured preparations with amylase did not affect the staining of this carbohydrate complex.

Further identification was attempted with the ultraviolet microscope. Sections were cut at 2 μ. They were deparaffinized with xylene and denatured in ethanol-formalin. Small pieces were mounted on quartz slides in glycerol. There was no absorption in the ultraviolet rays unless the sections were first denatured, because the undenatured material was dissolved by the glycerol. Denatured preparations absorbed heavily in the ultraviolet rays. A consistent series of curves (Fig. 2) was obtained when the modified extinction [15] ($\log \frac{I_0}{I}$) was plotted. These curves showed a high absorption at 2650 A. and at 2804 A. The data indicate the presence of purine- and pyrimidine-containing substances and of proteins containing cyclic amino acids.

COMMENT

It has been suggested by Cobb [11] that the glycoprotein ground substance of bone is organized in a highly polymerized state and that the degree of aggregation increases in association with calcification. This interpretation was made in part from the nature of the reaction of tissue sections with the periodic acid-leucofuchsin reagent. The intense staining reaction of young bone was taken to show many free reactive groups, which would be characteristic of a substance in a low degree of polymerization. On the other hand the low intensity of staining observed in older, more differentiated bone was said to arise from the unavailability of reactive groups, as would be characteristic of a highly polymerized substance.

It is probable that the protein of the matrix has an affinity for calcium as was shown to be the case for cartilage by Freudenberg and Gyorgy.[16] If one conceives of the ground substance as containing a high density of negatively charged macromolecules,[17] the reactions with cations, especially calcium, may be anticipated. Specifically, this binding power has been attributed to chondroitin sulfate and similar substances.[18]

The great plasticity of connective tissue ground substance was stressed by Gersh and Catchpole.[4] They suggested that alterations in the ground substance led to the elevation of the serum mucoprotein or polysaccharide level observed by Seibert,[19]

15. The term "modified extinction" is used because it is recognized that the absorption in these preparations may show deviations from the conditions of the Beer-Lambert law.

16. Freudenberg and Gyorgy: Ueber Kalkbindung durch tierische Gewebe II, Biochem. Ztschr. **115**:96, 1921.

17. Joseph, N.; Engel, M. B., and Catchpole, H. R.: Interaction of Ions and Connective Tissue, Acta biochim. & biophys., to be published.

18. Rubin, P. S., and Howard, J. E.: Histochemical Studies on the Role of Acid-Muco-Polysaccharides in Calcifibility and Calcification, in Transactions of the Second Conference on Metabolic Interrelations, New York, Josiah Macy Jr., Foundation, 1950, p. 155.

19. Seibert, F. B.; Seibert, M. V.; Atno, A. J., and Campbell, H. W.: Variation in Protein and Polysaccharide Content of Serum in the Chronic Diseases, Tuberculosis, Sarcoidosis and Carcinoma, J. Clin. Invest. **26**:90, 1947. Seibert, F. B.; Pfaff, M. L., and Seibert, M. V.: Serum Polysaccharide in Tuberculosis and Carcinoma, Arch. Biochem. **18**:279, 1948.

Winzler,[20] Shetlar [21] and their associates in cancer, tuberculosis, arthritis, and in a number of other diseases. They reasoned that in these disease states, or even under certain normal circumstances, the glycoprotein polymers of the ground substance are broken down by enzymes produced by the connective tissue cells. The diffusible depolymerized fragments enter the blood, where they are detected as an elevation of the serum mucoprotein level. Catchpole [6] subsequently showed that the connective tissue about tumors in mice contained appreciably greater amounts of water-soluble, alcohol-insoluble mucoprotein when the blood mucoprotein levels were elevated. Also, high serum mucoprotein levels in scorbutic guinea pigs were reported by Pirani and Catchpole.[22] This evidence of connective tissue plasticity suggested that similar phenomena could be exposed in bone.

The histochemical evidence shows that alteration of the glycoprotein constituents of bone and of epiphysial cartilage follows the administration of parathyroid extract. The increase in intensity of staining, the presence of fibrous remnants of bone and of cartilage, and the intercellular aggregates of glycoprotein are believed to be the evidences of the depolymerization of the glycoprotein of bone matrix. This view is supported by the demonstration of greater amounts of alcohol-insoluble, water-soluble moieties in the bones of animals receiving parathyroid extract. It appears that the inorganic elements are released concurrently with the depolymerization of the ground substance, and the two are often associated in the phagocytosed intracellular aggregates. This total loss of bone substance under the influence of parathyroid extract was noted by McLean and Bloom.[2b] The connective tissue cells in the neighborhood of bone and cartilage spicules are affected directly or in some indirect manner by the parathyroid extract.[12]

The manner in which the glycoprotein matrix is depolymerized is not understood. We would suggest the following series of events: The connective tissue cells around the bone and the cartilage liberate enzymes which catalyze the reactions favoring the depolymerization of the glycoprotein of bone and of cartilage. As the protein polymer disaggregates and dissolves, the mineral-binding capacity is also reduced and calcium and phosphate are liberated or removed. In other instances of bone resorption, while the trigger mechanisms may differ, the same fundamental reactions in the bone ground substance are envisioned.

The histochemical observations refer to isolated bones, while the serum mucoprotein values are presumed to reflect, in a cumulative manner, the liberation of degraded bone ground substance from the entire skeleton. Because of this the serum mucoprotein level might be considerably elevated in the face of small morphological and histochemical changes in individual bones.

The hormone effect apparently continues for many hours after administration, but carbohydrate-containing casts do not appear in the kidney until very large doses

20. Winzler, R. J., and Smyth, I. M.: Studies on the Mucoproteins of Human Plasma II, J. Clin. Invest. **27**:617, 1948.

21. Shetlar, M. R.; Foster, J. V.; Kelly, K. H.; Shetlar, C. L.; Bryan, R. S., and Everett, M. R.: Serum Polysaccharide Level in Malignancy and in Other Pathological Conditions, Cancer Res. **9**:515, 1949.

22. Pirani, C. L., and Catchpole, H. R.: Serum Glycoproteins in Experimental Scurvy, A. M. A. Arch. Path. **51**:597 (June) 1951.

have been used. These plugs, in all likelihood, correspond to the eosin-staining material reported by Chown and associates [23] in their animals.

Circumstantial evidence suggests that the carbohydrate-protein or polypeptide complex in the kidney is derived from the blood mucoprotein and therefore may ultimately represent in part a residue of bone and cartilage matrix. The purine- and pyrimidine-containing substances may come from osseous tissue degradation, renal damage, or from some other sources. Preliminary studies of urine from the parathyroid-extract-treated animals indicated the presence of substances reacting with orcinol and with the tyrosine reagent. The possibility that this material is related to the urinary mucoproteins demonstrated by Tamm and Horsfall [24] should be considered. A comparison of the concentrations of this substance in urines from normal and from hormone-treated animals is now in progress.

Some of the tubular mucoprotein deposits contain inorganic phosphate-carbonate and therefore, presumably, calcium. While not all the renal calcification occurring in hyperparathyroidism is of this type, some stones may be formed in this manner. It is indeed possible that in other instances of renal lithiasis calcification occurs through an interaction between glycoprotein and inorganic ions. It would be of considerable interest to learn whether persistent elevation of the plasma mucoprotein level in other circumstances predisposes to the occurrence of kidney stones.

While no systematic attempt was made to determine the relation between the rise of the plasma mucoprotein level and the dose of parathyroid extract, there seems to be a relationship which deserves further investigation, since the method may have merit for the assay of the hormone.

CONCLUSIONS

Parathyroid extract administered to rats causes depolymerization and solution of the glycoprotein ground substance of bones and of the cartilage spicules at the epiphysis.

There is an associated rise in the serum mucoprotein level which appears to be related to the degree of bone change and roughly to the dose of parathyroid extract.

Very large doses of the hormone cause severe renal damage, notably blocking of the tubules by mucoprotein, which sometimes contains phosphate-carbonate, presumably as a calcium salt. Ultraviolet-ray-absorption studies of the casts indicate the presence of purine- and pyrimidine-containing substances and of proteins containing cyclic amino acids.

The effect of parathyroid extract on calcium and phosphorus metabolism may arise through an influence on the state of the mucoprotein of bone and cartilage.

In this investigation, Dr. H. R. Catchpole and Dr. I. Gersh gave counsel, Mr. Henry Finck assistance in making the ultraviolet measurements, and Mrs. Eleanor Wolatz technical assistance.

23. Chown, B.; Lee, M.; Teal, I., and Currie, R. J.: On Experimental Production of Nephritis in Rats by Means of Parathyroid Hormone and of Vitamin D, J. Path. & Bact. **49**:273, 1939.

24. Tamm, I., and Horsfall, F. L.: Characterization and Separation of an Inhibitor of Viral Hemagglutination Present in Urine, Proc. Soc. Exper. Biol. & Med. **74**:108, 1950.

STUDIES ON HYPERSENSITIVITY ARTERITIS IN RABBITS

I. Effect of Acute Arteritis on Blood Pressure
II. Observations on Animals Subjected to Numerous Large Injections of Horse Serum

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SINCE the time of Gull and Sutton the relationship between small blood vessel disease and high blood pressure has been under more or less continuous investigation. This relationship of hypertension and arteritis has been reviewed recently by Corcoran, Page, Masson, Taylor and Dustan.[1] The present investigation was undertaken to learn what effect, if any, hypersensitivity arteritis experimentally produced in rabbits would have on the blood pressure.

METHODS

Hypersensitivity was produced in the rabbits by intravenous injections of sterile horse serum without preservative,[2] a dose of 10 cc. per kilogram of body weight being used. A schedule of two injections similar to that described by Rich and Gregory[3] was employed in all instances except for the long-term experiments, in which a monthly injection schedule was used in one instance and a semimonthly schedule in the other.

Blood pressure was determined by the method of Grant and Rothschild,[4] and the results were recorded as mm. of Hg of systolic pressure in the central artery of the ear. Although the pressure is lower in the central artery of the ear than in the carotid artery, the reproducibility was such as to provide reliable evidence of the degree and the duration of an alteration of pressure.

All rabbits were obtained from the same colony, and all weighed between 2 and 3 kg. at the beginning of the experiment. No attempt was made to select the animals except by observing them during the control period to determine their general state of health.

During the two long-term experiments described below, the urine of each animal was tested twice weekly for blood by the benzidine method, and all positive results were confirmed by ether extraction.[5] At the same time, the urine was tested for protein by the use of Exton's reagent after centrifugation of the specimen.[5]

During both of these experiments, each animal was tested for the Arthus phenomenon. At the end of the experiments, a precipitin titer[6] was determined on the blood of each animal

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This work was supported by a grant from the United States Public Health Service.

1. Corcoran, A. C.; Page, I. H.; Masson, G. M. C.; Taylor, R. D., and Dustan, H.: A. M. A. Arch. Int. Med. **87**:732 (May) 1951.

2. The horse serum was supplied by Sharp & Dohme Inc., Glenolden, Pa.

3. Rich, A. R., and Gregory, J. E.: Bull. Johns Hopkins Hosp. **72**:65, 1943.

4. Grant, R. T., and Rothschild, P.: J. Physiol. **81**:265, 1934.

5. Kolmer, J. A., and Boerner, F.: Approved Laboratory Technique, Ed. 4, New York, D. Appleton-Century Co., Inc., 1945.

6. Martin, D. S.: J. Lab. & Clin. Med. **28**:1477, 1943

except those dying unexpectedly and from which blood was not available. An autopsy was performed on each of the animals, and brain, heart, lungs, liver, pancreas, spleen, adrenals, kidneys, gonads, urinary bladder, skeletal muscle, esophagus, stomach, intestines, and mesentery were examined macroscopically and microscopically.

The sites of sampling and the number of blocks taken from the principal organs were standardized after the preliminary experiment, so that approximately comparable material from each animal was seen. The heart was sectioned throughout at approximately 4-mm. intervals perpendicularly to the septum, and all blocks were embedded. A single block was taken from each lung near the hilum and perpendicularly to it. Blocks were taken from each major lobe of the liver near the entrance of vessels and perpendicularly to them. Near the root of the mesentery a block was taken perpendicularly to the vessels. Three to four blocks of small intestine, one of stomach and one to two of colon were taken. Two blocks extending through the cortex and the medulla were taken from each kidney. In the long-term experiments, the mesentery was rolled and sectioned perpendicularly to the vessels. The kidneys from the animals in the long-term experiment were sectioned perpendicularly to the hilum at approximately 4-mm. intervals. Blocks were taken at random from the other organs.

PROCEDURE

Five separate but related groups of experiments were performed to determine the relationship between arteritis and blood pressure, and also to observe the effect of certain factors on the incidence of arteritis.

The first group of experiments was undertaken to explore the suitability of the blood pressure method and the effectiveness of the horse serum method in producing arteritis. All six of the rabbits used for this experiment were females and had been on a whole-oat diet for several weeks. All of them had been previously used for Friedman pregnancy tests, and all had shown positive results. Each animal was given 10 cc./kg. of sterile horse serum intravenously. A second injection was made 17 days later, and the animals were killed on the 27th day. Since this procedure resulted in arteritis of varying degrees in five of six rabbits without elevation of blood pressure, it was decided to perform a long-term experiment to determine whether alterations of blood pressure would occur if the experiment were extended over several months.

In the long-term experiment it was decided to use the monthly-injection schedule mentioned by McKeown.[7] Six rabbits were given 100 cc. of 7% gelatin in 0.85% saline per kilogram intravenously once each month, and these animals served as controls. Twelve rabbits were given 10 cc. of sterile horse serum per kilogram intravenously each month and served as the experimental animals. These animals died in acute anaphylaxis as follows: one at 2 months, two at 3 months, three at 5 months and three at 6 months. The final group of three experimental animals and the controls were killed at 7½ months. Blood pressures were taken three times each week, and the urine was examined for protein and blood twice a week. Only one arterial lesion was found in each of four of the experimental animals. These lesions occurred in animals dying at 2, 5, 6, and 7 months. They were similar in all, regardless of the difference in time between two months and seven months.

Since the first long-term experiment produced only a few lesions, it was decided to perform a second experiment of six months' duration in which semimonthly injections were substituted for the monthly injections. Eight control rabbits and 14 experimental rabbits were treated as in the previous experiment except that the injections were twice as frequent. The injections were given more slowly in this experiment, so that only two animals were lost before the end of the experiment (one at three months, one at four months). Only two arterial lesions developed. Hence, it was decided to return to short-term experiments to extend the original observations.

The fourth experiment was designed to use two groups of animals. One group was pretreated intravenously with 30 cc. of urine from a pregnant woman to simulate the pretreatment of the first group of animals; the other group had no pretreatment. Both groups of animals were given 10 cc. of sterile horse serum per kilogram by intravenous injection three days after the one group had received the urine intravenously. The incidence of lesions was higher in both of these groups than in the long-term experiments, but was the same in the two groups.

7. McKeown, E. F.: J. Path. & Bact. **59**:547, 1947

Since the animals used in the first experiment had been on a whole-oat diet for several weeks before sensitization, it was decided to repeat this procedure to determine whether this diet was a factor in the production of lesions. One group of seven animals was placed on the oats diet two weeks before the first injection was given. The other group was given the regular diet of rabbit chow. The injections of horse serum were on the same schedule as in Experiment 4. Again the frequency of lesions was high and similar in both groups.

RESULTS

Because of the marked difference in the incidence of arterial lesions between the short- and the long-term experiments, the results of the two types of investigation will be described separately.

Short-Term Experiments.—In the three short-term experiments, 12 animals were on a full diet of rabbit chow and had had no special treatment before or during the sensitization; 12 other rabbits were also on a full diet but had had 30 cc. of human pregnancy urine intravenously before sensitization; 7 rabbits had had only a whole-oat diet for two weeks before and during the sensitization. As seen in Table 1, the distribution and the intensity of the lesions did not vary significantly between

TABLE 1.—*Frequency of Lesions in Hypersensitive Rabbits Treated Only with Horse Serum as Compared with Those Treated with Human Pregnancy Urine and Horse Serum and Those Treated with a Whole-Oat Diet and Horse Serum*

Site and Nature of Lesions	Number of Rabbits in Which Lesions Were Observed		
	Horse Serum Only	Human Pregnancy Urine and Horse Serum	Oats Diet and Horse Serum
Heart			
Endocarditis and myocarditis	10 of 12	9 of 12	4 of 7
Arteritis	7 of 12	8 of 12	2 of 7
Arteritis in one additional organ	3 of 12	4 of 12	0 of 7
Arteritis in more than one additional organ	2 of 12	3 to 12	1 of 7

these groups, indicating that none of the three procedures altered the reaction to sensitization.

The lesions observed in these short-term experiments were the same as those described by other investigators and included panarteritis, endocarditis, and focal myocarditis. The panarteritis occurred in a segmental distribution and principally in large muscular arteries. There was considerable variation in lesions within the same animal and among animals killed after elapse of the same postinjection time interval. In some instances the lesion was characterized by an extensive cellular exudate in the adventitia and perivascular tissues. A similar but less extensive change was seen in the subendothelial tissues, and the two exudates were connected by a narrow band of exudate in the media. In these lesions "fibrinoid" change was uncommon, and the exudate was composed principally of lymphocytes and histiocytes with scattered eosinophiles and neutrophiles (Fig. 1). In those sites where the lesion was more marked, the exudate was more extensive in the media and subendothelial regions, and there commonly was "fibrinoid" change in the adventitia (Fig. 2*A*). It was only in the most severe lesions that the media was involved by a fibrinoid change. In some arteries the lesions were evidenced principally by fibro-

blasts present in the intima, with a few lymphocytes scattered throughout the intima and the media and with prominent plasmacytes and histiocytes in the adventitia (Fig. 2*B*).

The endocarditis occurred in both the mural and the valvular endocardium. In the mural endocardium it was characterized by an intense infiltration of lymphocytes, histiocytes, and a few neutrophiles just under the endothelium without other visible reaction. The valvular lesions were similar, but in addition there was an occasional thickening of the whole leaflet or cusp. This thickening consisted of a network of fine, pale pink fibers, with only occasional fibrocytes (Fig. 3). The lesions of the myocardium were characterized by focal collections of lymphocytes without other visible change. These lesions were not seen to be associated with vessels.

Fig. 1.—Photomicrograph of an artery from the base of the mesentery of Rabbit 1738, showing extensive adventitial and intimal exudates joined together by three foci of exudate in the media. The exudate is principally lymphocytes and histiocytes with scattered eosinophiles and neutrophiles. Hematoxylin and eosin stain; × 79.

To determine the relationship between blood pressure and acute arteritis, animals were grouped according to the degree of vascular disease, and the mean arterial blood pressure for each of the groups was plotted against time in days. The symbol *H. S.* on the abscissa indicates the time of injection of either horse serum or gelatin. In Figure 4 there are three groups: (1) all animals that had acute arteritis; (2) all animals receiving horse serum but showing no arteritis (animals with only endocarditis or myocarditis are included in this group); (3) all animals receiving gelatin rather than horse serum. It can be seen that both of the groups

given horse serum had a blood pressure above that of the controls at the beginning of the experiment. The blood pressure then dropped somewhat and again rose toward the end of the experiment. The maximum difference between the controls and the animals with arteritis occurs at the end of the experiment and is then a difference of only 8 mm. Hg. It should be noted also that the final mean blood pressure of the group with arteritis is only 4 mm. Hg above the initial mean pressure.

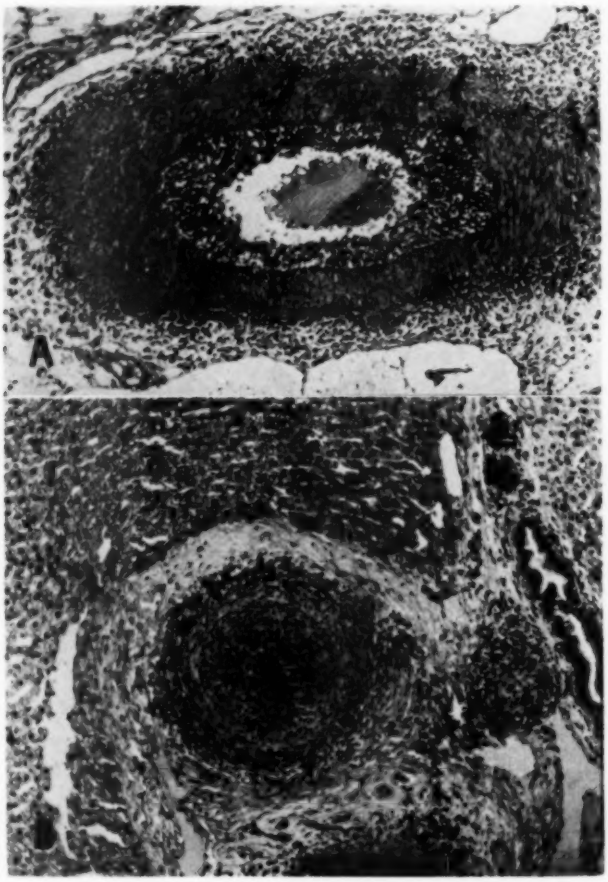

Fig. 2.—*A*, photomicrograph of an artery from the base of the mesentery of Rabbit 1751, showing extensive exudate in the intima, the media, and the periarterial tissues and a band of fibrinoid change extending throughout the adventitia. Hematoxylin and eosin stain; × 79.

B, photomicrograph of a blood vessel of the liver of Rabbit 1751, showing a prominent proliferation of fibroblasts in the intimal connective tissue and a moderate infiltration of lymphocytes and plasmacytes within the vessel wall and the surrounding tissue. This is probably the active phase of a lesion such as that depicted in Figure 5*A*. Hematoxylin and eosin stain; × 79.

Long-Term Experiments.—The two long-term experiments gave similar results and will be reported together. The control animals when tested for the Arthus

phenomenon all had negative reactions, and precipitin tests of their blood with gelatin were negative. The experimental animals when tested for the Arthus phenomenon with horse serum all had positive reactions, and the 15 from which blood was available all had positive precipitin tests except one animal that died in acute anaphylaxis. The incidence of lesions is presented in Table 2. As seen in Table 2, the 14 controls

TABLE 2.—*Frequency of Lesions in Control Rabbits Treated for Periods Ranging to Seven Months with Large Intravenous Injections of Gelatin as Compared with the Experimental Rabbits Receiving Horse Serum by Intravenous Injection*

	Arteritis	Endocarditis	Myocarditis	Hepatitis	Nephritis
Controls	0 of 14	0 of 14	0 of 14	3 of 14	4 of 14
Experimental rabbits	6 of 26	14 of 26	17 of 26	24 of 26	17 of 26

Fig. 3.—Photomicrograph of the mitral valve of Rabbit 1752, showing extensive edema and focal exudative endocarditis. The exudate is composed of lymphocytes, plasmacytes, and a few histiocytes. Hematoxylin and eosin stain; × 79.

revealed no arteritis, endocarditis, or myocarditis. There was a low incidence of chronic periportal hepatitis and interstitial nephritis, but the degree of infiltration in all lesions was very slight in comparison with that in experimental animals.

In the 26 experimental animals, the most noteworthy observation was the scarcity of arterial lesions. No acute lesions were found, and of the six chronic

lesions noted, four were in the mesentery and two in the heart. The changes ranged from a slight infiltration of lymphocytes and plasmacytes in periarterial connective tissue to intimal and periarterial connective tissue proliferation with lymphocytic exudate, but without disruption of the internal elastic lamina (Fig. 5*A*). The endocardial and myocardial lesions were similar to those previously described in the short-term experiment (Fig. 5*B*). In addition to these

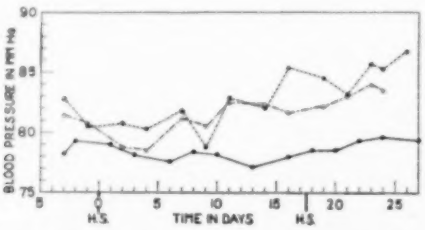

Fig. 4.—The solid black line represents the mean systolic pressure of 12 rabbits given gelatin rather than horse serum and serves as a control level of blood pressure for a two-injection experiment. The interrupted lines represent the animals receiving horse serum. The interrupted line with solid dots represents the mean blood pressure of those animals in which arteritis was found, while that with open dots represents the mean blood pressure of those animals in which no lesions, or lesions other than arteritis, were found.

cardiovascular lesions, slight to moderate infiltrations of lymphocytes and plasmacytes were frequently seen in the periportal spaces of the liver, occasionally with slight connective tissue proliferation (Fig. 6*A*). A similar cellular infiltration was common in the interstitial tissues of the kidney. These were most common in the region of the arcuate vessels (Fig. 6*B*) but in the more advanced lesions were found throughout the cortex as well. No glomerular lesions were identified in any of the animals.

TABLE 3.—*Comparison of Mean Blood Pressures of Control Rabbits, Treated for Periods Ranging to Seven Months with Large Intravenous Injections of Gelatin, and Experimental Rabbits, Treated Similarly with Horse Serum* *

	Mean Blood Pressures													
	Preinjection							Pretermination						
Control	78	78	78	76	78	80	80	80	80	82	82	82	82	80
Experimental	76	78	76	74	76	76	78	82	80	82	82	82	80	82

* The mean of the last seven blood pressures before starting the injections and the mean of the last seven before terminating the experiment are given; no significant variation occurred between these extremes.

Since the vascular lesions produced in these animals were so slight and the blood pressure alterations so insignificant, the results of the blood pressure determinations will be summarized (Table 3). The mean blood pressures for the seven determinations immediately before the first injection of horse serum and the mean blood pressures for the last seven determinations on the surviving animals before the termination of the experiment are tabulated for both control and experimental animals. The intervening readings are omitted since they show no variation from the two extremes presented here.

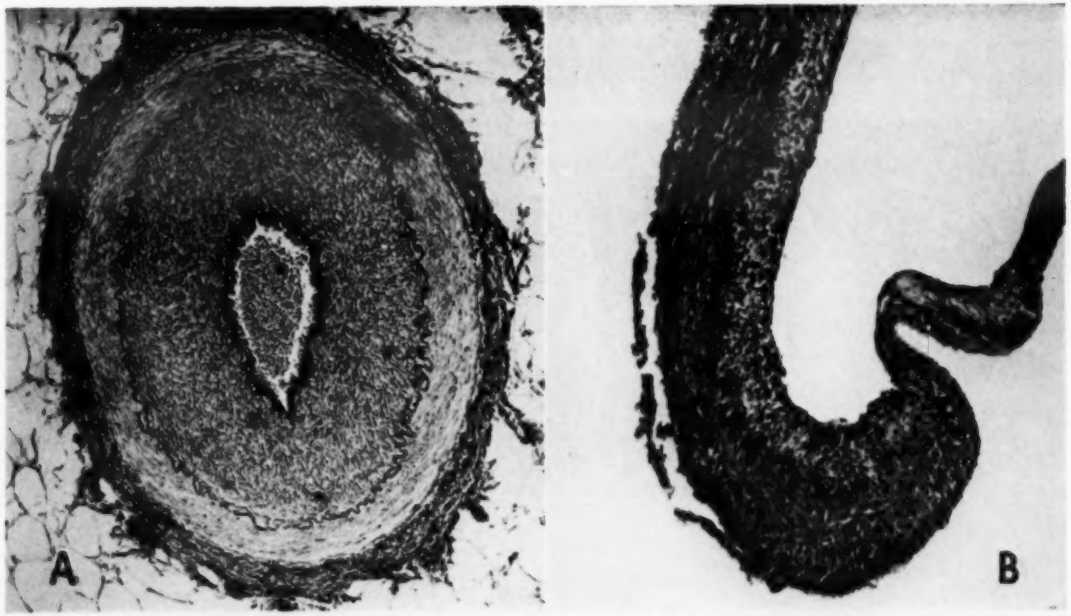

Fig. 5.—*A*, photomicrograph of a mesenteric artery from Rabbit *22*, which died two months after the beginning of the experiment. There is extensive fibroblastic proliferation in the intima. The only exudate is a rare lymphocyte in the intima. Elastic-Van Gieson stain; × 79.

B, photomicrograph of the mitral valve of Rabbit *63*, which was killed six months after the beginning of the experiment. There is extensive exudation of lymphocytes and histiocytes beneath both the ventricular and the atrial surfaces of the valve. Hematoxylin and eosin stain; × 79.

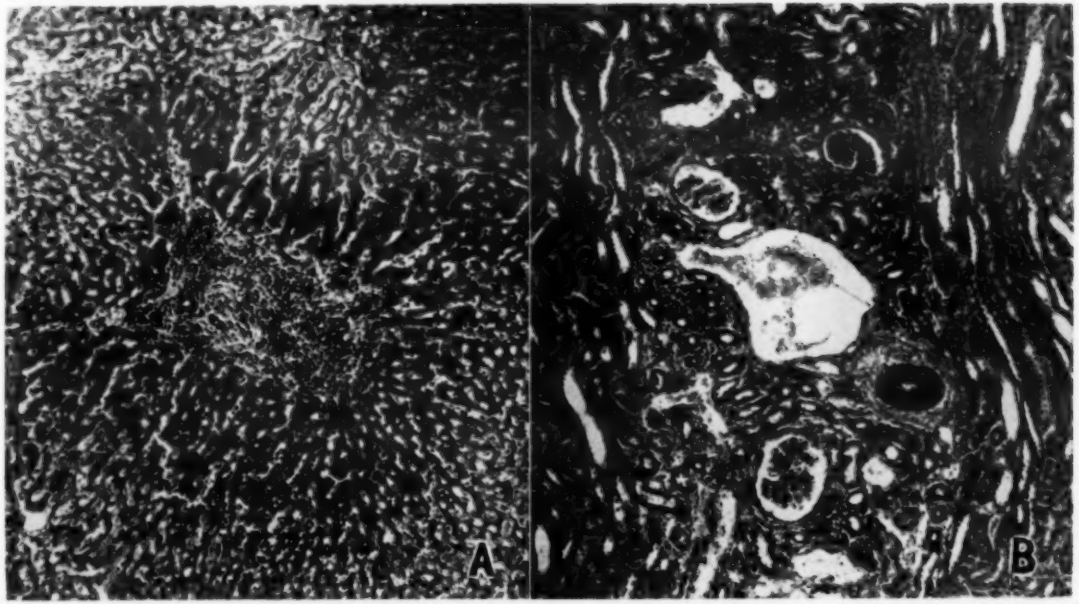

Fig. 6.—*A*, photomicrograph of liver of Rabbit 15, which died five months after the beginning of the experiment. The periportal exudate of lymphocytes and plasmacytes is characteristic. Slight periportal fibrosis has occurred. Hematoxylin and eosin stain; × 79.
B, photomicrograph of kidney of Rabbit 17, which died six months after the experiment began. The interstitial infiltration of lymphocytes and plasmacytes near the arcuate arteries as seen here was a common observation in the long-term experiments. Hematoxylin and eosin stain; × 79.

COMMENT

Short-Term Experiments.—The present work was undertaken to gain information concerning the relationship between hypertension and acute necrosis of arteries by studying the blood pressures of rabbits in which acute disseminated arteritis had been induced by horse-serum hypersensitization. While this work was in progress, Smull, Wissler, and Watson [8] reported a study of the blood pressures of three rabbits with definite arteritis, three with slight or no arteritis, and three treated with salicylate, and found no significant change in the pressures. They stated that their results confirmed the findings of Hopps and McCollum.[9] The results of our investigation show a slight rise in the mean arterial blood pressure of the animals given horse serum, and this rise was greatest in those rabbits having arteritis. However, in the group showing the greatest change, the mean increase was only 8 mm. Hg over that of the control group. Such an increase is not considered to be indicative of hypertension and may be only a reaction to the presence of necrosis and inflammation.

Since acute necrosis and inflammation of arteries occurred without significant hypertension in these experiments, hypertension cannot be postulated as a factor of the development of the acute lesions in the arteries of rabbits sensitized to horse serum.

Long-Term Experiment.—It was hoped that by repeatedly injecting large amounts of horse serum into rabbits over a period of six to seven months, progressive vascular lesions would be obtained and observations could be made on the blood pressure throughout this period. However, the procedure failed to produce arteritis except for one chronic lesion observed in each of six rabbits. Because of the inadequacy of the lesions, the observation that no alteration occurred in the blood pressure of these animals is of no value in furthering our knowledge of the relationship between arteritis and blood pressure.

Since the technique of sampling for histologic study was the same for the long experiments as for the short ones, the relative scarcity of arterial lesions in the long experiments must be a real one. It would then appear that the arterial lesions either failed to develop or developed and regressed during the long experiments. The latter possibility seems more likely, since a few vessels were found in which fibroblastic proliferation was prominent and varying amounts of mononuclear exudate persisted. These lesions are similar to those observed by Hawn and Janeway [10] in animals killed four weeks after a single injection of bovine serum or bovine albumin. It would appear probable that the lesions occurred in our animals with the original injection of horse serum but that further injections failed to produce significant progression of the arterial injury and that the lesions already present gradually decreased in activity. Such findings suggest that the reaction of the animals to horse serum changed during the course of the experiment so that the later injections of horse serum failed to produce the degree of arterial injury originally invoked, and that the lesions gradually subsided, some even going on to healing. On the other hand, the production of endocardial lesions was similar to

8. Smull, K.; Wissler, R. W., and Watson, J. M.: J. Lab. & Clin. Med. **33**:936, 1948.

9. Hopps, H. C., and McCollum, W. C.: Federation Proc. **6**(Pt. II):394, 1947.

10. Hawn, C. V., and Janeway, C. A.: J. Exper. Med. **85**:571, 1947.

that in the short-term experiments, indicating that the reaction of the endocardium did not diminish.

The principal lesions noted in these animals consisted of slight to moderate endocarditis, myocarditis, periportal hepatitis, and interstitial nephritis. These lesions are similar to those reported by Longcope [11] in his study of chronic anaphylaxis. Even though similar hepatic and renal lesions occurred in control animals, they were so much less common and so much less severe that the hepatitis and nephritis of the experimental animals are considered as a possible result of the hypersensitivity.

Rich and Gregory [2] and More and Waugh [12] have reported glomerulonephritis in short-term experiments with large injections of horse serum, and McLean, Fitzgerald, and Younghusband [13] have reported proliferative glomerular lesions with daily injections of small amounts of horse serum over a period of three to thirteen months. We have not seen lesions of the glomeruli in any of our material, and in the long-term experiments we have been unable to demonstrate either blood or protein in the urine of the animals. We are unable to explain this variation in results, unless it is due to unknown differences in the rabbits.

SUMMARY

No significant elevation of blood pressure was observed in rabbits sensitized to horse serum, even though acute necrotizing and inflammatory lesions of arteries occurred.

Attempts to influence the susceptibility of sensitized rabbits to arterial damage by previous injection of hormone (Friedman pregnancy tests) or by alteration of diet (whole-oat diet) were unsuccessful.

In rabbits receiving repeated injections of large amounts of horse serum over several months, only rare arterial lesions were observed, whereas endocardial lesions were prominent. The arterial lesions were all in various stages of healing, whereas the endocardial lesions were still exudative. This suggested the possibility that the arteries of these animals became less reactive to the antigen with repeated injections but that the reactivity of the endocardium remained unchanged.

The lesions of endocarditis, myocarditis, hepatitis, and interstitial nephritis produced in the long-term experiments were comparable to those previously reported.

The failure to demonstrate glomerular lesions in the kidneys of any of these animals is unexplained.

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11. Longcope, W. T.: J. Exper. Med. **18**:678, 1913.

12. More, R. H., and Waugh, D.: J. Exper. Med. **89**:541, 1949.

13. McLean, C. R.; Fitzgerald, J. D. L., and Younghusband, O. Z.: A. M. A. Arch. Path. **51**:1 (Jan.) 1951.

STUDY OF THE DEATH OF IRRADIATED AND NONIRRADIATED CELLS BY TIME-LAPSE CINEMICROGRAPHY

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THE DEGENERATION and the death of cells exposed to x-rays and other reagents have been studied previously by bright-[1] and dark-field [2] microscopy, by the method of unstained-cell counts, and by stained histologic section.[3]

Peculiar structures termed primary vacuoles were observed in rabbit lymphocytes which had been irradiated in vitro and incubated at 37 C. for three to five hours. The vacuoles were best seen with dark-field microscopy and appeared as small, round, dark, single or multiple areas usually surrounded by thick, brightly illuminated walls. With bright-field illumination, the vacuoles were translucent and distorted the shape of the cell. In fact, the highly irregular shape was the predominating feature of the cells. In stained smears and in tissue sections, the primary vacuoles were seen to be intranuclear and acidophilic. When eosin or safranine was added to an irradiated and incubated suspension, the cells with primary vacuoles usually failed to stain and were therefore considered to be viable. In short, after irradiation and incubation the lymphocyte had one or more vacuoles in the nucleus, was highly irregular in shape, and was still viable.

After 20 hours of incubation, nearly all of the irradiated cells were dead, as indicated by their stainability with dyes. The dead cells had pyknotic nuclei, which stained deeply and uniformly with safranine. On dark-field illumination the pyknotic nucleus appeared dark and was termed a "secondary" vacuole, which differed

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1. Schrek, R.: (*a*) Studies in Vitro on the Physiology of Cells: Histologic Reactions of Living Tissues to Hypotonic Solutions, Am. J. Path. **21**:1101-1112, 1945; (*b*) Studies in Vitro on the Physiology of Cells: Factors Affecting the Delayed Cytocidal Action of X-Rays, J. Cell. & Comp. Physiol. **28**:277-304, 1946.

2. Schrek, R.: (*a*) Dark Field Observations on Lymphocytes Exposed to X-Rays and Other Injurious Agents, Proc. Soc. Exper. Biol. & Med. **64**:381-384, 1947; (*b*) Primary and Secondary Vacuoles in Thymic Cells Exposed in Vitro to X-Rays, J. Cell. & Comp. Physiol. **30**:203-224, 1947.

3. Schrek, R.: Cytologic Changes in Thymic Glands Exposed in Vivo to X-Rays, Am. J. Path. **24**:1055-1065, 1948.

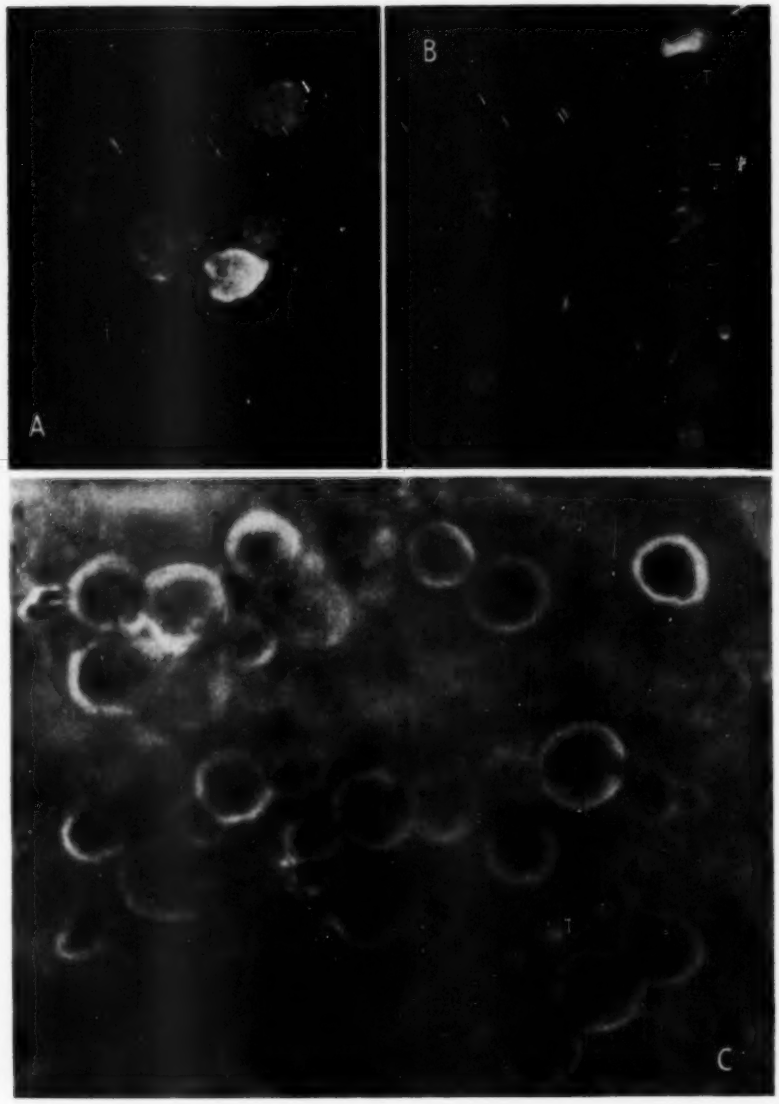

Fig. 1.—*A*, cells of a rat lymphosarcoma, reticulum cell type, under dark-field microscopy. The print was made from a 16-mm. motion-picture film. The cells are large, round, with numerous fine granules in the cytoplasm and the nucleus. One cell with bright, coarse granules and a bright nuclear wall is probably dead.

B, cells of the rat lymphosarcoma which have been incubated in vitro at 37 C. for five hours. The cells are irregular in shape, lobulated, and have one or several dark vacuoles.

C, cinemicrographic view of irradiated lymphocytes after 80 minutes' incubation. The cells are round and have peripheral bright fine granules. The background and the interior of the cells are dark.

in appearance from the previously described primary vacuole. The secondary vacuoles were relatively large, round, and bounded partly by a thick, brightly illuminated line and partly by a crescent of fine and coarse granules. On staining, the crescent of granules was found to be in the cytoplasm of the cell.

Incubation of control, nonirradiated, suspensions also resulted in lymphocytes with primary and secondary vacuoles. The rate of production of vacuolated lymphocytes was, however, much slower in the nonirradiated than in the irradiated suspensions.

About half of the cells in the nonirradiated suspensions died without the development of primary vacuoles or pyknotic nuclei. In fact, the dead cells retained more or less the normal cytologic structures, including the nuclear wall and the chromatin granules. They appeared to have undergone death by a process akin to fixation. Under dark-field illumination these dead "fixed" cells had many fine or coarse nuclear granules and usually had a thick, bright nuclear wall.

Apparently different physicochemical processes were responsible for the death of lymphocytes. One process led to cells with primary vacuoles and pyknotic nuclei. A different reaction resulted in dead cells with "fixed" nuclei.

Similar unpublished studies were made on suspensions of a transplantable rat lymphosarcoma, reticulum cell type.[4] The tumor cells under dark-field microscopy were large, round, and had numerous fine bright granules in both the nucleus and the cytoplasm (Fig. 1*A*). In some cells the granules in the nuclei were less bright than those in the cytoplasm. The nucleus was large, round, without a visible nuclear wall. The cytoplasm was sparse. As can be seen in Figure 1*A*, a few cells were more brilliantly illuminated and had coarse granules. These cells were believed to be dead, because they were similar in appearance to cells treated with a fixative.

On incubation of the suspensions, the untreated tumor cells underwent primary vacuolation with marked irregularity of shape and with the development of multiple vacuoles (Fig. 1*B*). Evidently, in these cells the degenerative changes were similar to those seen in the irradiated and nonirradiated lymphocytes.

In spite of the qualitative similarity of the degenerative changes of the cells of the rat lymphosarcoma and the cells of the rabbit thymus, there were quantitative differences. Cells with primary vacuoles developed more rapidly and were more numerous in suspensions from the tumor than in those from the thymus. Furthermore, the tumor cells did not survive as long in vitro as the rabbit lymphocytes.

In the previous work the degenerative processes were studied by examining successive batches of cells. In this study the morphologic changes in the same group of cells were observed by the method of cinemicrography.

METHODS

Cellular suspensions were prepared by the methods described in a previous publication.[1b] Cells from the rabbit thymus were suspended in a medium consisting of equal parts of homologous serum and phosphate-Ringer's solution. The suspension was then subjected to 1,000 r of x-rays. Cells from the transplantable reticulum cell sarcoma of the rat were suspended in human serum and were not irradiated. The suspensions were used to prepare, as in tissue

4. Two rats with the tumor were received from Dr. Anna Goldfeder. The tumor arose spontaneously and was first transplanted by Dr. Halsey J. Bagg. A description of the tumor has been published by Dr. Goldfeder (Proc. Soc. Exper. Biol. & Med. **59**:104-109, 1945).

culture, a hanging drop composed of equal amounts of chicken plasma and cellular suspension. The depression of the slide was flooded with human serum or diluted rabbit serum. The cover slip with hanging drop was inverted over the hollow of the slide and sealed with paraffin.

The cinemicrographic equipment was rigidly mounted in a quiet basement to eliminate vibrations. It consisted of a 35-mm. motion-picture camera connected to a microscope by means of a split-beam eyepiece. The microscope was equipped with a phase condenser, a 4-mm. objective, and a 10× ocular. The microscopic lamp had a 6-volt ribbon filament. Camera, microscope and lamp were enclosed in a lucite* incubator maintained at 37 C. Accessories of the motion-picture camera permitted the taking of a 3-second exposure at 15-second intervals. The 35-mm. film was developed as a negative, and 16-mm. prints were made for projection.

The motion pictures were studied in detail by repeated projection in order to observe and to time the morphologic changes in the cell. Each second on the movie screen was equivalent to four minutes in the life of the cell, and one minute corresponded to four hours.

For more detailed study of the cells and for more precise timing of the processes, a large series of prints were made on photographic paper. As the negative film had consecutive numbers at the side of every sixteenth frame, it was possible to determine the exact number of frames between the prints and to calculate the time intervals with accuracy.

Slight movements of the cell or of its parts could be seen more definitely on the projection of the motion picture than by studying successive prints. To study these subtle phenomena, drawings were made of the cells in successive prints, and the drawings were carefully superimposed. By this method, slight movements could be readily demonstrated and studied.

OBSERVATIONS

Irradiated Lymphocytes After 80 Minutes' Incubation.—In one film taken of irradiated lymphocytes of the rabbit, about 20 cells were in good focus in the photographic field 80 minutes after incubation (Fig. 1*C*). With phase microscopy the cells appeared bright against a dark background. The cells were round or oval, with fine granules peripherally and dark centers. The nuclei could not be made out. The appearance of the lymphocytes in this preparation was somewhat similar to that obtained with dark-field microscopy, although the fine granules were not so numerous or as well illuminated.

On projection of the film, the cells shown in Figure 1*C* were actively mobile and were therefore considered to be alive. The mobility of the cells is illustrated in the superimposed drawings of Figure 2. This figure shows the location of some of the cells at four-minute intervals during incubation from 140 to 156 minutes.

It is seen in Figure 2 that one cell showed amoeboid movement, i. e., movement more or less in one direction. In the entire film only a few cells showed amoeboid motion. The amoeboid cells usually were round or oval but were sometimes moderately elongated in the direction of movement.

Several cells shown in Figure 1*C* and in the projected film seemed to move back and forth within a narrow zone, or moved into and out of focus repeatedly. This vibratory motion was in all probability an active process of the cell.

A third type of movement, illustrated in Figure 2, was seen in a few cells. These remained in the same location but showed minimal movements of the cell outline. Even drawings at 30-second intervals showed these slight changes in shape of the cell. In the projected film, the cell wall appeared to have a rapid wavering or undulating motion, which was particularly prominent in cells with dark interiors.

The mobility of the living cell has been divided into amoeboid, vibratory, and undulating motion merely for descriptive purposes. These three types of movement do not necessarily represent three distinct functional activities. Presumably all

the cells had undulating movements, although the more dramatic amoeboid and vibratory movements usually overshadowed the subtler but more fundamental undulations of the cell wall.

Irradiated Lymphocytes After 16.3 Hours of Incubation.—After incubation for 16.3 hours, nearly all the cells had undergone considerable change. The cells were irregular or lobulated in shape, with irregularly distributed fine granules and indistinct cell outlines. On projection of the film all the cells except one were seen to be completely immobile. The rigid immobility was proof of the death of the

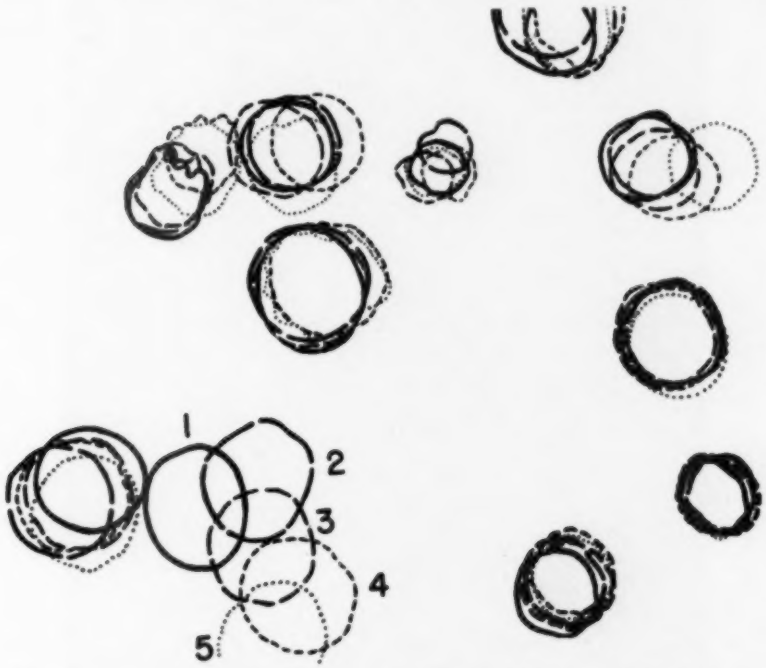

Fig. 2.—Superimposed drawings of irradiated lymphocytes that had been incubated 140, 144, 148, 152, and 156 minutes (*1* to *5*, respectively). The cells show amoeboid, vibratory, and undulating movements.

cells and was also an indication of the absence of vibration in the microscope and the camera.

Cell Death.—It seems reasonable, then, to use active mobility as a criterion of viability and immobility as a sign of death. The manner in which the cell was transformed from mobility to immobility or from life to death was of particular interest in this study. By repeated projection and careful study of the film, 17 cells were observed to suffer this transformation, and one cell remained alive after 24 hours of incubation. There seemed to be one well-defined moment when most cells were seen to lose their activity. The time of death could therefore be deter-

mined and the duration from the onset of incubation calculated with accuracy. Some cells seemed to lose their active mobility but continued to show slight movements. These apparently passive movements were either the result of the activity of adjacent living cells or were due to surface-tension phenomena within the dead protoplasm. In such cases the change from active to passive mobility was sometimes not as clearcut as the complete loss of movement. With experience, it seemed that

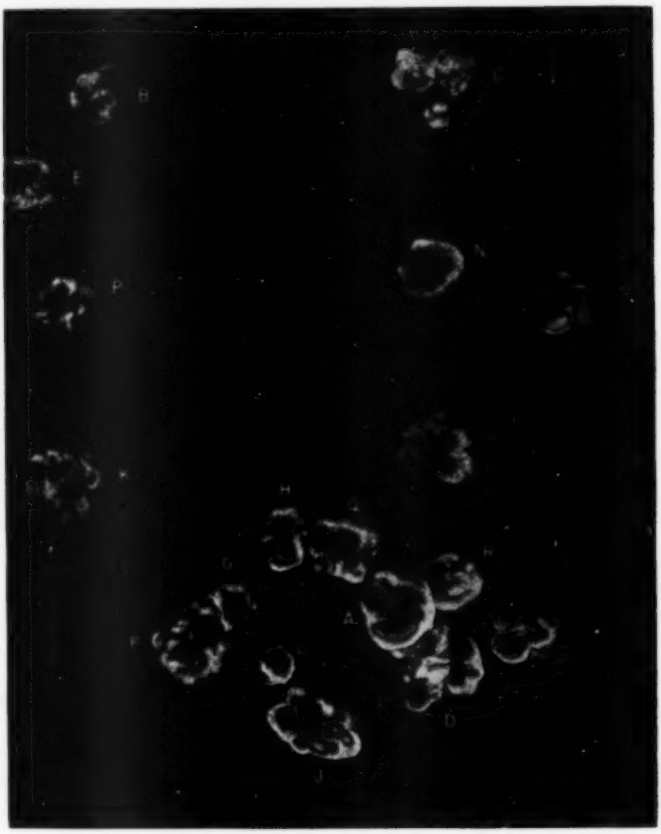

Fig. 3.—Superimposed drawing showing location and shape of irradiated lymphocytes at the time of the death of the cells. The drawing also shows cell *R*, which remained viable after 24 hours of incubation. The cells are lettered in the order of the time of their death.

even in these cells the time of death could be determined with a fair degree of accuracy.

By means of the prints of the film, a composite drawing (Fig. 3) was made of 17 cells shortly after the time of death. This figure shows the location and the shape of the cells shortly after death. The cells are lettered from *A* to *Q* in the order of their death. Cell *R* in the figure was not dead after 24 hours of incubation.

Does the death of one cell affect its neighbors? Is there any pattern in the order of death? These questions are difficult to answer, since the figure presents the cells in only one plane. It is seen, however, that cells *F*, *G*, and *H* were adjacent to each other and died in that order in a period of 20 minutes. Aside from these cells no relationship could be seen between the time of death and the location of the cells.

A curve showing the period of survival of the 18 cells in the film is presented in Figure 4. For comparison, the figure also presents a similar curve of the survival of irradiated lymphocytes as determined in a previous study by the method of

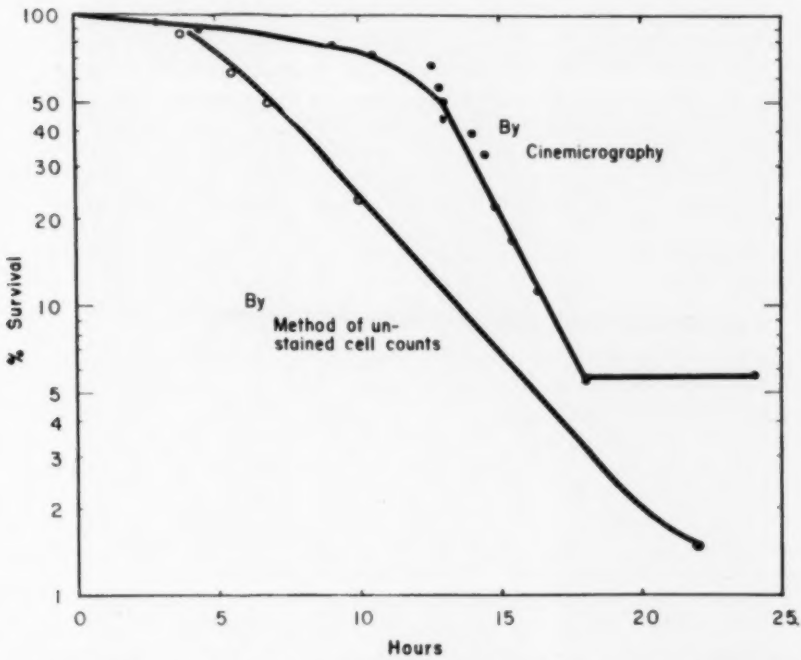

Fig. 4.—Survival curves of irradiated lymphocytes according to a cinemicrographic film and according to the method of unstained-cell counts. The curve labeled "by cinemicrography" is based on the survival of 18 cells.

unstained-cell counts. It is seen that the two curves differ both in shape and in slope. The 50% survival of the irradiated cells was 13 hours according to this cinemicrographic study and 7 hours according to the method of unstained-cell counts. Since the two experiments were done at different times and with different methods, the variation in the curves is not surprising. The difference in the two curves suggests the need for further work.

Death by Lobulation or Vacuolation.—Cell *K* in Figure 3 may be used to illustrate the changes which occurred at death. For many hours this cell appeared normal (Fig. 5*A*). It was round or oval, with three large bright granules, probably

mitochondria, which moved actively in the cytoplasm. The cell outline showed rapid undulating movements. As the cell was near the edge of the photographic field, it occasionally moved in and out of the field as a result of vibratory and amoeboid motion. After 13.8 hours of incubation the cell was still round (Fig. 5*A*). Five minutes later the cell was irregular and lobulated (Fig. 5*C*). The lobulations were deep-seated, persistent, and showed progressive development. In Figure 5 and in the projected film the rapid changes in shape of cell *K* continued for 17.5 minutes; then the cell became more or less stationary (Fig. 5*J*). However, slow minor changes continued, and the final shape was not achieved until 78 minutes after the onset of lobulation (Fig. 5*L*).

In previous work, similar changes in shape were observed (compare Fig. 2 of a previous publication[1b]). With dark-field illumination the changes were observed to be the result of the development of primary vacuoles. In the present study, with phase microscopy, the detailed structure of the cells and the nucleus could not be demonstrated adequately. It seemed evident, however, that the changes of shape observed in this study were associated with the development of primary intranuclear vacuoles.

The end-result of the degenerative changes in cell *K* was an immobile, irregular, flattened, dead cell. It was seen previously that the process of primary vacuolation resulted in pyknotic nuclei, which appeared as secondary vacuoles in dark-field microscopy. Although the phase microscope did not reveal the structure of the nucleus of the dead cell *K*, it is probable that it had a pyknotic nucleus.

The question arises, exactly when did cell *K* die? During the process of lobulation the cell showed rapid changes in shape, so that it was difficult to state whether there were any undulating movements. Some cells, however, showed definite vibratory movements during the stage of lobulation. In addition cells with intranuclear vacuoles have been found previously to resist staining with eosin and safranine. It would seem, then, a cell may be considered viable during the stage of rapid change of shape.

In most cells the period of lobulation was followed immediately by complete immobility of the cell. In cell *K*, however, the stage of active lobulation was followed by slow, minimal changes. These were considered to be passive movements and were not considered to be a sign of viability.

Of the 17 dead cells in Figure 3, all except 4 (*A*, *B*, *H*, and *N*) were seen to undergo a period of rapid lobulation before or during death. The duration of the process was determined by running the film both forward and backward. The time of lobulation was found to vary from 10 to 36 minutes, with an average of 19.7 minutes and a standard deviation of 7.4 minutes.

It is evident from this study that the death of most irradiated lymphocytes was not instantaneous but that they suffered considerable morphologic change before they became immobile and dead. Therefore, this type of death is termed chronic death with lobulation or vacuolation.

Other Types of Death.—A different type of death was observed in the projected film of the irradiated lymphocytes. A few cells, as cell *A*, showed a sudden cessation of their undulating and vibratory movements. This change was frequently associated with a slight change from the round or oval contour of the living cell to a slightly irregular, polygonal shape. By use of superimposed drawings, it was

noted that this loss of mobility and change in shape took place from one frame of the movie to the next. In other words, this type of death occurred within 15 seconds and therefore may be termed acute.

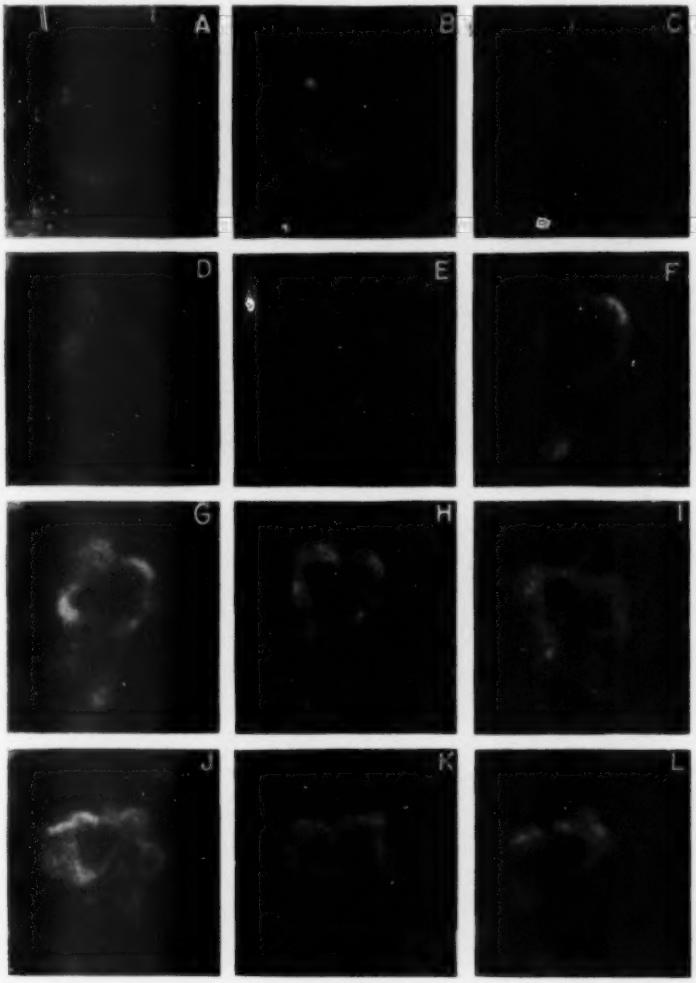

Fig. 5.—Cinemicrographic views of the degeneration and death of cell *K*. The first picture was taken after 13.8 hours of incubation. The successive pictures were made at 1, 3, 5, 6, 8, 9.5, 11, 13.5, 17.5, 29, and 78 minutes after the first picture.

It is not possible to state definitely what the nuclear structure of cell *A* was after its acute death. Fixatives are known to kill cells rapidly or acutely, and the dead cells have well-preserved nuclear detail. In addition, previous work has shown

that after incubation of cellular suspensions some of the dead cells have normal nuclear structure. Cell *A* and other cells suffering acute death probably retained to a considerable extent their normal cytologic structure.

A third type of morphologic change associated with cell death could be recognized affecting cell *N* in Figure 3 and a few other cells in other films. This cell gradually became sluggish and showed slow undulating movements. It also began to show slight, superficial irregularity of shape. The irregularity was not deep-seated as in cell *K* but seemed to involve chiefly the cytoplasm as in the lymphocytes of Figure 1 of a previous study [1b] and as in the macrophage of Figures 6 and 7 in this study. The death of cell *N* may have been the result of cytoplasmic changes.

Lymphosarcoma.—Several motion pictures of cells of the lymphosarcoma were prepared. On projection of the films, the cells appeared large, round, and at first had active undulating and vibratory movements. A few cells were in amoeboid motion. After a short period of incubation the mobility of the cells decreased in rapidity but the cells continued viable.

After a few hours of incubation, many cells began to show multiple, extensive lobulations, which changed rapidly. The duration of the lobulations was not timed, since the film showed relatively few malignant cells. It seemed, however, that the duration of the degenerative stage of lobulation was in the same order of magnitude for the tumor cells as for the irradiated lymphocytes.

One movie of a suspension of a reticulum cell sarcoma showed the degeneration of a large cell which could not be definitely recognized but which may have been a macrophage. At the beginning of incubation and photography this cell was spindle-shaped and large (more than 70 micra long and 12 micra wide, Fig. 6*A*). A print of the movie showed only one thin, elongated protoplasmic projection. The nucleus was large, oval, and nongranular, with one or two fairly large, dark nucleoli. At the two poles of the nucleus were two masses of numerous, fairly coarse cytoplasmic granules, which in the movie showed active agitation.

The cell did not show any motility, and there was no undulating movement of the wall of the body of the cell. The protoplasmic projection, however, showed considerable activity for approximately two hours. The projection had a slight but rapid side-to-side and up-and-down whip-like motion. In addition it tended to retract partially and then to extend again. When partially retracted, the end was usually fan-shaped with rapid undulating movements of the fimbria.

After 148 minutes of incubation the protoplasmic projection started to retract, forming a thick bulbous end (Fig. 6*B*). Meanwhile the body of the cell thickened and became triangular in shape. The cytoplasmic granules continued to be in agitation, usually moving along the long axis of the cell. A thin zone of clear ectoplasm was present beneath the cell wall.

In four and a half hours the protoplasmic projection was completely retracted and the cell was rounded (Fig. 7*A*). The nucleus was oval and appeared somewhat smaller than at first. The cytoplasmic granules almost completely encircled the nucleus and continued to be active. The ectoplasm was visible. The cell wall seemed to be quiescent. From 4.5 to 6.7 hours the cell showed no change in shape. The movements of the granules increased in tempo, and the nucleus slowly rotated.

A new process began 6.8 hours after the onset of photography (Fig. 7*B*). Blebs developed over the entire cell wall, causing considerable superficial irregu-

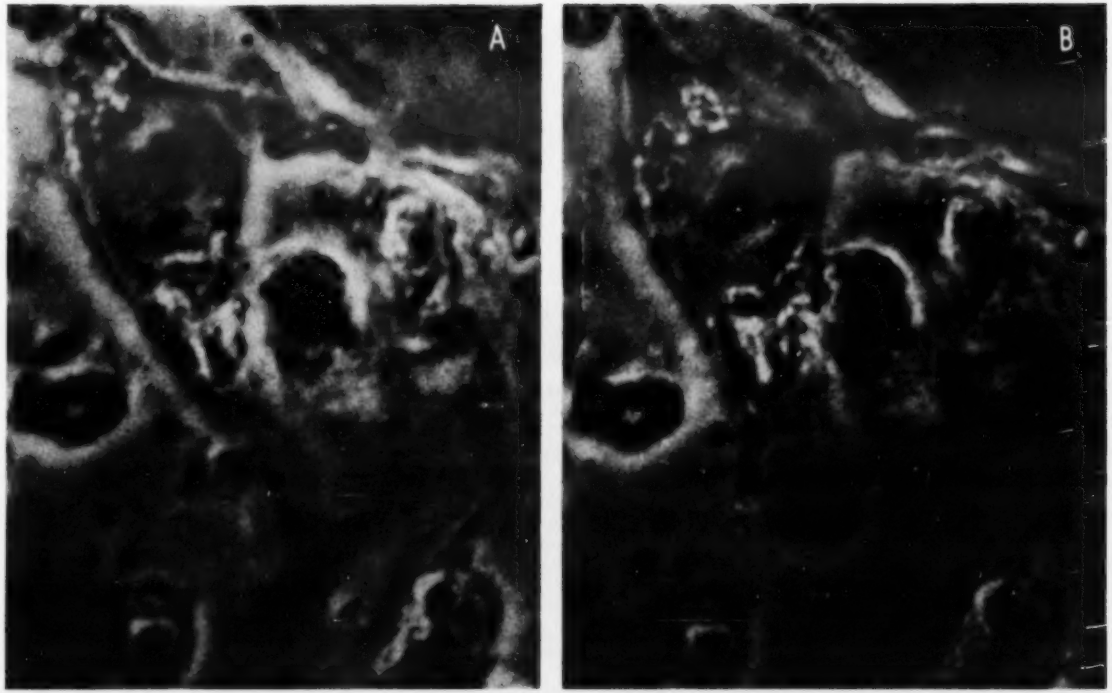

Fig. 6.—Cinemicrographic views of a cell (macrophage?) in a suspension of the rat lymphosarcoma. *A*, after 66 minutes of incubation. The cell has an apparently normal nucleus, nucleoli, cytoplasmic granules, and a protoplasmic projection with a fimbriated, undulating edge. *B*, after 148 minutes. The protoplasmic projection has partly retracted.

larities in the shape of the cell. The blebs seemed to be confined to the cytoplasm and showed rapid changes in appearance, so that differences could be seen in successive frames taken 15 seconds apart.

About six minutes after the onset of bleb formation, fine granules developed in the nucleus (Fig. 7*C*). The granularity started at the periphery and spread rapidly, reaching the nucleolus within four minutes.

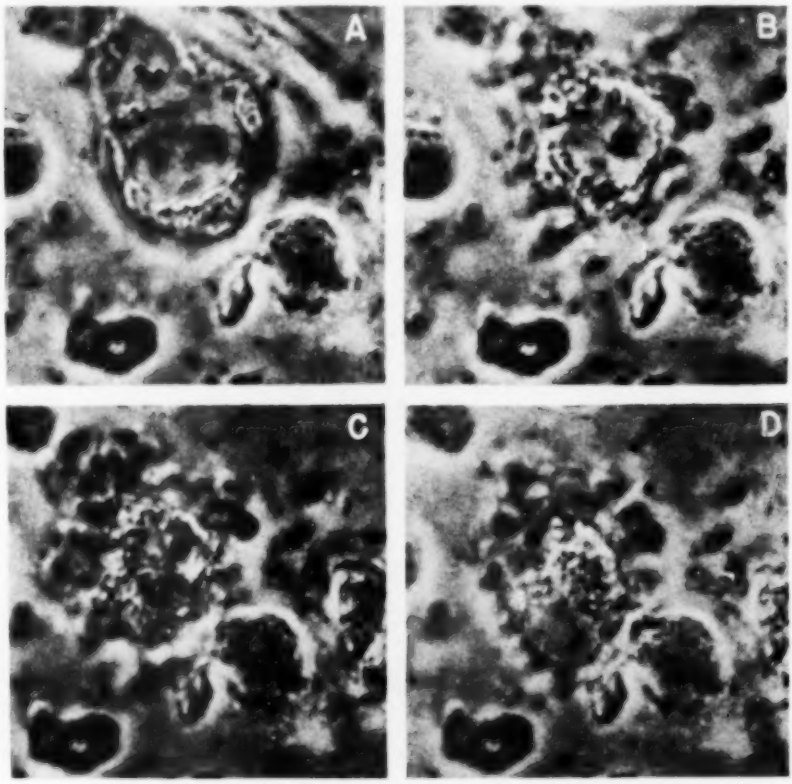

Fig. 7.—Further cinemicrographic views of the cell shown in Figure 6 after continued incubation: *A*, after 276 minutes. The cell is rounded up. *B*, after 408 minutes. The cytoplasm has many blebs, and the peripheral zone of the nucleus is granular. *C*, after 411 minutes. The nucleus is almost completely granular. The cytoplasm continues to show bleb formation. *D*, after 419 minutes. The cell has a granular nucleus and a large vacuole, which has probably been extruded from the nucleus.

At this time there appeared a large, clear vacuole, which was probably nuclear. The vacuole rapidly increased in size and was soon extruded from the nucleus (*7D*). Meanwhile the blebs continued to develop at a slow rate until gradually the activity quieted down and, seven hours after the onset of incubation, the cell became completely motionless.

At the present stage of knowledge concerning cell degeneration it is not possible to explain the various changes in the cell. It is only possible to describe the process and to continue to accumulate data on different types of cells and on different causes of death.

Cinemicrography.—Cytopathology or the manner in which cells react to toxic reagents has been studied by several investigators using cinemicrographic methods. Costero [5] and Kolle, Laubenheimer, and Vollmar [6] observed the reactions of microglial cells in tissue culture. Costero found that pilocarpine when added to a culture killed the cells but when injected into the cell caused only a temporary cessation of motion. He concluded that pilocarpine acted on the surface of the cell. Curare killed the cells in culture, but morphine accelerated cell movements. The effects of other alkaloids were studied. Hughes [7] investigated the effects of many reagents on cells in division. Aminopterin [8] (4-aminopteroylglutamic acid) and colchicine inhibited the formation of the mitotic spindle during metaphase. In addition, iodoacetamide inhibited the reconstruction of the nuclei in telophase. Lettré,[8] also, studied the effect of mitotic poisons and demonstrated the film at the Fifth International Cancer Congress. Although considerable work has been done in cytopathology by cinemicrography, additional study in this field seems indicated.

The effect of radiation on cells was first studied by Canti and Spear.[9] They exposed tissue cultures to radium and photographed the cultures by means of bright-field illumination. By this method, they demonstrated that radium caused a decrease in the number of cells undergoing mitosis. The bright-field illumination did not permit a good demonstration of the structural changes in the cell.

According to Kolle, Laubenheimer, and Vollmar,[6] Costero found that moderate doses of x-rays had no toxic effects on cells of rat sarcoma. Larger doses of x-rays and cathode rays caused retraction of pseudopodia and granularity and death of the cells.

Buchsbaum and Zirkle [10] used cinemicrography to study the degenerative changes in irradiated erythrocytes from Amphiuma and from man. These cells were subjected to alpha particles from polonium. The cells first shrank, then swelled and hemolyzed.

Stroud and Brues [11] showed that moderate doses of tritium oxide did not kill cells immediately but caused prolongation, abnormalities, and inhibition of mitotic

5. Costero, I.: Experimenteller Nachweis der morphologischen und funktionellen Eigenschaften und des mesodermischen Charakters der Mikroglia, Ztschr. ges. Neurol. u. Psychiat. **132**:371-406, 1931.

6. Kolle, W.; Laubenheimer, K., and Vollmar, H.: The Importance of Cinematography in Biological Researches and Especially in Cells and Cell Cultures, Internat. Rev. Educational Cinematography **4**:749-759, 1932.

7. Hughes, A. F. W.: The Effect of Inhibitory Substances on Cell Division: A Study of Living Cells in Tissue Cultures, Quart. J. Micr. Soc. **91**:251-277, 1950.

8. Lettré, H.: Division cellulaire et poisons de la mitose, presented to the Fifth International Cancer Congress, 1950, p. 25.

9. Canti, R. G., and Spear, F. G.: The Effect of Gamma Irradiation on Cell Division in Tissue Culture in Vitro, Proc. Roy. Soc., London, s. B **105**:93-98, 1929-1930.

10. Buchsbaum, R., and Zirkle, R. E.: Shrinkage and Swelling After Alpha Irradiation of Various Parts of Large Erythrocytes, Proc. Soc. Exper. Biol. & Med. **72**:27-29, 1949.

11. Stroud, A. N., and Brues, A. M.: The Effect of Tritium (H^3) on Cells Cultivated in Vitro, ANL-4451: 111-117, U. S. Atomic Energy Commission, 1950.

division. Cell death occurred only during or after mitosis.[1] Larger doses of the radioactive hydrogen killed directly resting cells, which exploded and extruded cytoplasmic granules with a shrinkage of the nucleus.

Classification of Cell Death.—In spite of in vitro and in vivo studies by many investigators, cytopathology does not provide as yet sufficient data on the mechanism of the action by which reagents affect cells. Without knowledge of the cellular structure or the physiologic process which is affected by the cytotoxic agents, it is not possible to develop an adequate classification of cell death. It might be useful, however, to review the data available.

One important type of cell death is that resulting from nuclear vacuolation. In irradiated suspensions, cells with primary vacuoles were numerous and prominent, according to dark-field studies by Schrek.[2b] Warren, Holt, and Sommers [12] demonstrated that irradiated tissues fixed by freezing and drying have many cells with intranuclear vacuoles. Radiosensitive cells were observed by these investigators to have large intranuclear vacuoles which apparently exploded from the nuclei with lethal effect. The radioresistant cells also were found to have intranuclear vacuoles which subsided or regressed in 24 hours after irradiation. It would appear from these studies that one method of cell degeneration is the formation of intranuclear vacuoles.

Schrek [2b] observed vacuoles not only in irradiated lymphocytes but also in nonirradiated ones. In the present study, the process of vacuolation or lobulation in irradiated lymphocytes was seen to last on the average only 20 minutes. As a result of their transient character and as a result of their unsatisfactory preservation by fixatives, the intranuclear vacuoles are not conspicuous in ordinary histologic sections. However, in routine biopsies an occasional cell does have these structures and an occasional biopsy specimen has been found to have a relatively high percentage of cells with intranuclear vacuoles.

The best understood type of cell death is seen in the fixation of cells with preservation of nuclear and cytoplasmic structures. The mechanism of death is the denaturation and coagulation of proteins both in the nucleus and in the cytoplasm. The death of cells has been studied by many different methods. This type of cell death was indirectly studied by investigators who searched for an ideal fixative which would preserve the structures of the living cell. These workers usually checked the fixatives by stained smears and, more recently, by electron microscopy. Dark-field microscopy of tissue cultures was used by Strangeways and Canti [13] to study cells killed by fixation. These investigators showed that fixatives produced shrinkage and distortion of the cells. They concluded that none of the fixatives is entirely satisfactory. Zollinger [14] used phase microscopy to study cells killed by fixatives. He found that these fixed cells have a "brilliant" nucleus

12. Warren, S.; Holt, M. W., and Sommers, S. C.: Some Early Nuclear Effects of Ionizing Radiation, Proc. Soc. Exper. Biol. & Med. **77**:288-291, 1951.

13. Strangeways, T. S. P., and Canti, R. G.: The Living Cell in Vitro as Shown by Dark-Ground Illumination and the Changes Induced in Such Cells by Fixing Reagents, Quart. J. Micr. Sc. **71**:1-14, 1927-1928.

14. Zollinger, H. U.: Cytologic Studies with the Phase Microscope: IV. Morphologic Changes Associated with the Death of Cells in Vitro and in Vivo, Am. J. Path. **24**:1039-1053, 1948.

which can be differentiated from the nucleus of normal cells. In the present cinemicrographic studies, some cells were seen to undergo acute death. Presumably this acute death is the same as cellular death by fixation.

Death of Irradiated Cells.—Duryee [15] ascribed cytoplasmic changes as the cause of death in amphibian cells exposed to x-rays. Buchsbaum and Zirkle [10] observed by cinemicrography that alpha-particle irradiation of human and Amphiuma erythrocytes caused a cytoplasmic reaction, with shrinkage and swelling of cells. On the other hand, Schrek [2b] and Warren, Holt, and Sommers [12] considered that x-rays caused nuclear changes in lymphocytes and other cells. In addition, the nuclei of cells in mitotic division are affected by x-rays (Sparrow [16]). What then is the primary effect of x-rays, nuclear or cytoplasmic?

The diverse claims concerning the effect of x-rays on cells are not necessarily contradictory. The nucleus of the lymphocyte and the dividing nucleus of many cells seem to be particularly radiosensitive and to react to small doses of radiation. On the other hand, large amounts of x-rays may act as a universal protoplasmic poison and affect the cytoplasm of all cells. Stroud and Brues [11] obtained visual evidence of the dual action of radioactive hydrogen. Moderate doses (1,000 rep) altered the behavior of cells in mitosis, while large doses (50,000 rep) killed resting cells. It may be concluded that x-rays have two distinct effects: Small doses cause specific changes in the nuclei of lymphocytes and dividing cells; large doses of x-ray apparently effect nonspecific changes in the cytoplasm and nuclei of resting cells.

The hypothesis of the dual action of x-rays suggests several corollaries. In the first place, the equal range of sensitivity of lymphocytes and dividing cells may indicate a common physiologic process in the nucleus of the lymphocyte and in the mitotic nucleus. Secondly, the specific action of small doses of x-rays on the mitotic and the lymphocytic nucleus may be quite different from the nonspecific action of large doses of x-rays on nearly all living cells. Presumably findings obtained on the nonspecific action of x-rays will not necessarily apply to the specific action.

SUMMARY

Time-lapse cinemicrography with the phase microscope was used to record the survival and the death throes of normal lymphocytes of the rabbit thymus and of malignant lymphocytes of a transplantable lymphosarcoma of the rat. The thymic cells were subjected to 1,000 r of x-rays before photography.

The viability of the cells was indicated, in the projected film, by undulating, vibratory, and amoeboid movements of the cells. Dead cells were characterized by rigid immobility, but an occasional dead cell showed passive movements or slow postmortem changes.

In one film, the death of 17 irradiated lymphocytes was observed and timed. A survival curve was obtained, with 9 of the 18 cells surviving 13 hours and one, 25 hours.

15. Duryee, W. R.: The Nature of Radiation Injury to Amphibian Cell Nuclei, J. Nat. Cancer Inst. **10**:735-795, 1949.

16. Sparrow, A. H.: Radiation Sensitivity of Cells During Mitotic and Meiotic Cycles with Emphasis on Possible Cytochemical Changes, Am. N. Y. Acad. Sc. **51**:1508-1540, 1951.

An occasional cell died rapidly, as if killed by a fixative, with an abrupt loss of movement and a slight change in the shape of the cell. The death of a macrophage was characterized by formation of cytoplasmic blebs and subsequent development of nuclear granules.

Most of the irradiated and the malignant lymphocytes suffered a degenerative stage characterized by rapid changes in shape with formation of multiple lobulations. This degenerative stage lasted on the average 20 minutes and was followed by immobility and death of the cells.

With dark-field microscopy and with stained smears, the lobulation and distortion of shape of degenerating lymphocytes were observed to be associated with the formation of acidophilic primary vacuoles in the nuclei. The dead lymphocytes, after irradiation, were seen to have pyknotic nuclei.

It may be concluded that intranuclear vacuolation is a brief stage in the development of pyknotic nuclei in irradiated lymphocytes.

Case Reports

CEREBRAL NOCARDIOSIS

Report of a Case with Necropsy

NATHAN KAUFMAN, M.D.
AND
L. C. PRIETO Jr., M.D.
CLEVELAND

IN CASES of infection due to Nocardia asteroides it is believed that the organism gains entrance through the skin or through the respiratory tract. Apart from skin infection, most of the cases described have been those of pulmonary nocardiosis. When the organs were involved, the lungs or the bronchopulmonary lymph nodes were considered to be the primary site of the disease. Common metastatic sites are the brain and the meninges; in fact, central nervous system involvement was present in 12 of the 42 cases reported. It is of interest that in some of these 12 cases the most striking, the first and the only signs and symptoms were referable to the brain. The first human instance of Nocardia asteroides infection to be described[1] was just such a case, and at autopsy there were multiple brain abscesses from which Eppinger grew pure cultures of an acid-fast actinomycete, which he named Cladothrix asteroides. In addition, he observed the organism in a section of a bronchopulmonary lymph node.

In the case to be described here, the signs and symptoms were suggestive of a brain lesion, and it was not until craniotomy was performed that a diagnosis was made.

CLINICAL OBSERVATIONS

J. V., a 53-year-old white Hungarian male carpenter, was in apparently good health until one month prior to admission to the hospital, when he developed a "cold" with accompanying anorexia and malaise. He recovered, but 11 days before admission he became confused, disoriented, and restless and began to wander aimlessly around the neighborhood. He then stopped eating, and three days before admission he became increasingly drowsy, falling asleep easily, and developed a tendency to fall to the right when standing or walking. On the day of hospitalization he failed to recognize his family and became incontinent of urine. It was subsequently learned by the family that three months previously, while at work, he fell approximately 15 ft. (4.5 meters) off a scaffold but suffered no injuries. Approximately 20 years previously he fell off a scaffold and injured his eye and lip. On physical examination he was found to be confused as to time and place. His speech was incoherent, although he apparently understood requests and occasionally repeated words and parts of phrases. There was a slight weakness of the left side of the face. The left pupil was larger than the right, but both reacted to light and accommodation; the optic disks were hazy. The left rectus and cremasteric reflexes were hypoactive; otherwise the reflexes were normal. Kernig's sign was slight and questionable. The temperature on admission was 37 C. (98.6 F.), the pulse rate 80, and the blood pressure 160/90. A lumbar puncture had been performed one day before admission in the medical outpatient department, and

From the Departments of Pahology, Cleveland City Hospital and Western Reserve University School of Medicine.

1. Eppinger, Hans: Ueber eine neue pathogene Cladothrix und eine durch sie hervorgerufene Pseudotuberculosis, Beitr. path. Anat. 9:287-328, 1891.

at that time the spinal fluid pressure was 350 mm. of water; the Pandy test was 4 plus, and no cells were demonstrated. The blood Kline test gave a negative result. The white blood cell count varied from 20,000 to 30,000 per cubic millimeter; the differential count was normal on admission, but there was a shift to the left of the granulocytic series three days later. A roentgenogram of the thorax revealed a hazy, homogeneous density over the upper one-third of the left lung field, while skull roentgenograms revealed no abnormalities.

Soon after admission, bilateral temporal craniotomy was done; the dura did not bulge, and there was no evidence of abscess or hemorrhage. The spinal fluid pressure on that day was 520 mm. of water, and after 15 cc. of fluid was withdrawn, the pressure fell to 280 mm. of water. The following day the spinal fluid pressure was 580 mm. of water and fell to 190 mm. of water after withdrawal of 15 cc. of fluid. The patient became worse and comatose. Two days after admission there were relative hypotonia and hyperreflexia of the right lower extremity; Babinski's reflex was not present; a positive Brudzinski sign and a positive Hoffmann sign were obtained on the right. The left pupil was paretic and measured 5 to 6 mm. in diameter, while the right measured 3 to 4 mm. Three days after admission, bilateral occipital craniotomy was performed, and while an attempt was being made to needle the left lateral ventricle, pus was encountered. On the following day craniotomy was performed and a left parietal flap elevated. At this time a multiloculated abscess was located in the left parietal region and was dissected. Smears and cultures were made of the pus obtained from the last two procedures and the organism isolated was reported as Nocardia asteroides. The brain tissue examined histologically was a portion of the brain abscess, within which could be demonstrated branching filaments. In addition to the instillation of 25,000 units of penicillin and 5 mg. of streptomycin in 5 cc. of saline following the last operation the patient also received penicillin, streptomycin, aureomycin, and sulfadiazine. On the day of the last operation, four days after he was admitted to the hospital and 15 days after the onset of his symptoms, the patient died.

NECROPSY

There was a gaping defect in the brain over the left parietal lobe, posterior to the motor gyrus. This defect measured 8 by 4 cm. and was 3.5 cm. in depth. It was here that the abscess had been resected at operation (Fig. 1). In addition there were 13 abscesses scattered throughout the cerebral hemispheres. They varied from 1 to 3 cm. in diameter, were cystic, with soft, pale-yellow walls, and were filled with mucoid, glistening, odorless, light-green material. In addition, the right cerebellar hemisphere contained a single abscess measuring 3 cm. in diameter, which replaced a good deal of that hemisphere. Although there was no gross evidence of meningitis, a small abscess, measuring 1.4 cm., was found attached to the dura and pressed on the left occipital lobe.

Sections of the brain showed numerous abscesses composed predominantly of neutrophils, a few macrophages and lymphocytes, and several long, branching, thin filaments (Fig. 2). The latter measured less than 1 μ in diameter and were Gram positive and periodic acid-Schiff positive. The central portions of several abscesses were necrotic. Most of the abscesses were sharply demarcated from the surrounding brain tissue, but a few had ill-defined margins, and the adjacent tissue was markedly edematous and in some instances necrotic. Several small hemorrhagic foci were present about the abscesses. The subarachnoid space contained numerous granulocytes. The head and the body of the pancreas contained several abscesses, measuring from 1 to 4 cm. in diameter and filled with odorless, light-green, glistening, purulent material. At the hilus of the left lung there was one large bronchopulmonary lymph node, which measured 5 by 4 by 4 cm. This node was cystic and contained an abscess filled with glistening pus; the other

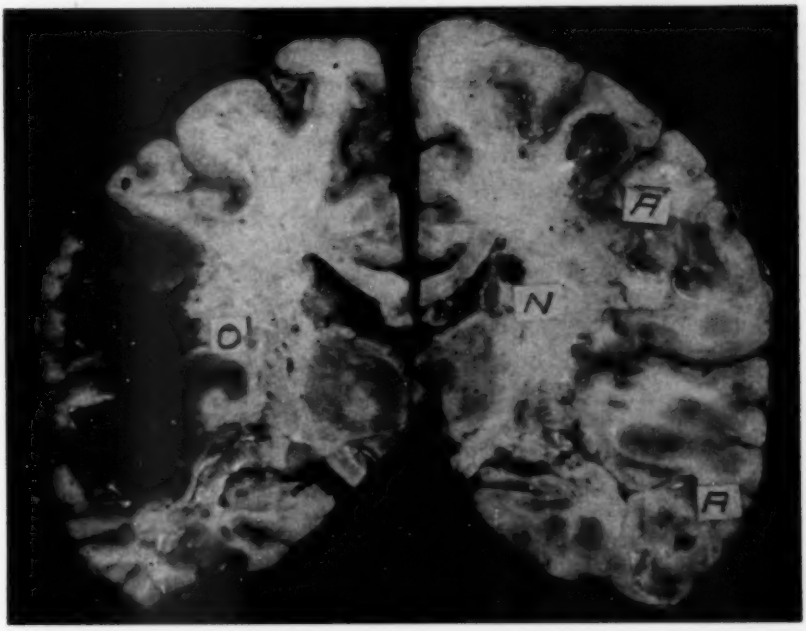

Fig. 1.—Coronal section of the cerebrum: *A*, abscesses of the right cerebral hemisphere. *O*, operative site. *N*, needle tract made at operation

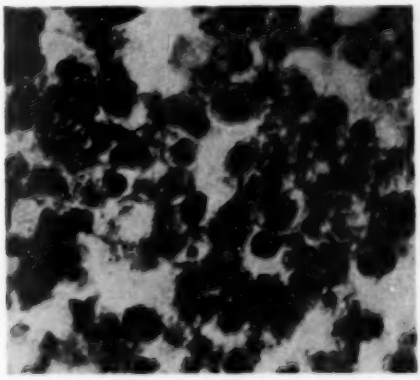

Fig. 2.—Section of brain abscess demonstrating the thin, branching filaments characteristic of Nocardia. × 980.

lymph nodes at the hilus were small and not remarkable. Microscopically, the pancreatic abscesses and that of the left bronchopulmonary lymph node were similar to those described in the brain, and the organisms were also demonstrated. There were numerous dense fibrous pleural adhesions between the lower portion of the upper lobe of the left lung and the chest wall. The greater portion of that lobe was firm and noncrepitant, and the cut surface was granular, gray streaked with white. There was an organizing pneumonia. Many of the alveoli were filled with fibroblasts, and the alveolar walls were thick. Other alveoli contained fibrin, granular acidophilic material, granulocytes, and macrophages with foamy cytoplasm. The bronchi also contained numerous granulocytes. Sections of lung stained by Gram's method, Verhoeff's method, and by the periodic acid-Schiff stain did not reveal any long, branching filaments similar to those seen in the brain, the pancreas, and the left bronchopulmonary lymph node. Sections of the other lobes of the lungs revealed chronic pulmonary emphysema and bronchopneumonia.

The anatomic diagnoses were multiple brain abscesses, pancreatic abscesses, lymphadenitis with abscess formation in a left bronchopulmonary lymph node—all due to Nocardia asteroides; organizing pneumonia in the upper lobe of the left lung; bilateral bronchopneumonia; chronic pulmonary emphysema; Laennec's cirrhosis.

MYCOLOGIC OBSERVATIONS

Moderate numbers of long, branching Gram-positive filaments were demonstrated in direct smears of the cerebral and pancreatic abscesses. Under aerobic conditions, cultures of the abscesses in the brain, the pancreas, and the left bronchopulmonary lymph node, on beef-heart-infusion agar enriched with 10% human blood, yielded small, white, dry colonies, measuring less than 1 mm. in diameter, after 48 hours' incubation at 37 C. The colonies later became thicker and slightly larger, and the medium was pitted. After eight days the colonies turned yellow and later orange. Growth was also obtained in beef-heart-infusion broth enriched with 3% human blood, and on Sabouraud's dextrose-agar medium and nutrient-agar slants. No growth was obtained on Littman's medium, with or without streptomycin. Gelatin was not liquefied, and no fermentation was obtained in cultures containing dextrose, lactose, sucrose, mannitol, maltose, inositol, dulcitol, rhamnose, and arabinose, but acid was present in cultures containing xylose after one month of incubation. On liquid media, growth occurred as a white surface pellicle. Smears showed delicate branching and interlacing filaments which were Gram positive and periodic acid-Schiff positive. These filaments were partially acid-fast, since they were decolorized by acid alcohol when stained with Verhoeff's carbolfuchsin, but many filaments remained red after decolorizing with 1% aqueous sulfuric acid.

COMMENT

In a recent review Tucker and Hirsch [2] collected 37 cases of nocardiosis, in 11 of which the central nervous system was involved. To these they added 3

2. Tucker, F. C., and Hirsch, E. F.: Nocardiosis, with a Report of Three Cases of Actinomycosis Due to Nocardia Asteroides, J. Infect. Dis. **85**:72-86, 1949.

cases of their own, in one of which there was a brain abscess. Hager, Migliaccio, and Young [3] reported 2 additional cases without cerebral involvement. In most of the cases of cerebral nocardiosis, lesions were also obvious elsewhere. Thus, the patient of Binford and Lane [4] first had an ischiorectal abscess, which was drained; then chronic suppurative pneumonitis and a massive brain abscess developed. The patient of Tucker and Hirsch [2] first complained of a suppurating wound of the right thigh. The case of Jacobson and Cloward [5] is more obscure in that, following symptoms of meningitis, Nocardia asteroides was grown from the spinal fluid. The patient recovered and gave the history that at one time he had a mass in the neck. The authors theorized that this was the primary focus. Again, in one of the cases described by Kirby and McNaught [6] there was a right cerebellar abscess, and although there was a small area of fibrosis with lymphocytes in the lung, no organism was demonstrated in the latter location. However, the authors speculated that this may have been the original site of the infection which had healed.

The present case resembles some of the previously described ones so far as the cerebral symptoms were the dominant ones. Here, too, one of the lungs showed evidence of old pulmonary inflammatory disease, and in view of the fact that a bronchopulmonary lymph node on that side was proved to be infected with Nocardia asteroides, we can assume that the lung or the bronchopulmonary lymph node was the primary site and hence that the organism metastasized to the brain. The pancreatic abscesses were essentially incidental findings, and the abscesses were probably of the same duration as those in the brain. Drake and Henrici [7] expressed the opinion that the disease originates in the lungs and then spreads to the brain. As in other cases, the abscesses presented a nonspecific appearance and, except for the demonstration of partially acid-fast mycelia, were not characteristic.

In view of the conflicting reports as to the management of the disease, the recent work of Runyon [8] is of definite value. He tested the sensitivity of the organism by both in vitro and in vivo methods and showed that in vitro aureomycin, streptomycin, and sulfadiazine were inhibitory, while terramycin, chloramphenicol, penicillin, and paraaminosalicylic acid were relatively ineffective. The in vivo studies showed penicillin and streptomycin to be ineffective and aureomycin of doubtful value. The only correlation between the in vivo and the in vitro studies was that sulfadiazine had been the most effective drug in the treatment of

3. Hager, H. F.; Migliaccio, A. V., and Young, R. M.: Nocardiosis: Pneumonia and Empyema Due to Nocardia Asteroides, New England J. Med. **241**:226-231, 1949.

4. Binford, C. H., and Lane, J. D.: Actinomycosis Due to Nocardia Asteroides, Am. J. Clin. Path. **15**:17-23, 1945.

5. Jacobson, J. R., and Cloward, R. B.: Actinomycosis of the Central Nervous System: A Case of Meningitis with Recovery, J. A. M. A. **137**:769-771 (June 26) 1948.

6. Kirby, W. M. M., and McNaught, J. B.: Actinomycosis Due to Nocardia Asteroides: A Report of Two Cases, Arch. Int. Med. **78**:578-591 (Nov.) 1946.

7. Drake, C. H., and Henrici, A. T.: Nocardia Asteroides: Its Pathogenicity and Allergic Properties, Am. Rev. Tuberc. **48**:184-198, 1943.

8. Runyon, E. H.: Nocardia Asteroides: Studies on Its Pathogenicity and Drug Sensitivities, J. Lab. & Clin. Med. **37**:713-720, 1951.

this disease. However, the authors cautioned that sensitivity studies should be done in all cases to determine the proper treatment, since the strain of the organism may vary.

SUMMARY

A case of nocardiosis is reported in which the main signs and symptoms pointed to the nervous system. Abscesses were found in the brain, in the pancreas, and in a left bronchopulmonary lymph node. Although Nocardia could not be demonstrated in the lungs either by examination of tissue sections or by culture, it is felt that the lungs were probably the primary site and that the organism had metastasized from there to the brain and the pancreas. Additional evidence for this supposition is that one of the left bronchopulmonary lymph nodes was diseased and Nocardia asteroides was demonstrated within it.

News and Comment

International Society for Geographic Pathology.—The Fourth Conference will be held in Liége, Belgium, July 15-18, 1952. The subject of the symposium is "Hepatitis" but one session of the Conference will be devoted to presentation of papers on other topics. For information, write to William B. Wartman, Secretary, National Committee for U. S. A., I. S. G. P., Northwestern University Medical School, 303 E. Chicago Ave., Chicago 11, Ill.

Announcement of Meetings.—The following meeting will be held at the Texas Medical Center, Houston, Texas, on April 25 and 26.

1. The Sixth Annual Symposium on Fundamental Cancer Research of the University of Texas M. D. Anderson Hospital for Cancer Research (subject—nutritional factors in cancer).

2. The Cancer Pathology and Radiology Conference of the University of Texas M. D. Anderson Hospital and Postgraduate School of Medicine (subject—tumors and other diseases of bone).

The South Central Section of the College of American Pathologists will participate in the Symposium and the Conference and hold their regional meeting on Saturday April 26.

Dr. M. M. Wintrobe, professor of medicine at the University of Utah is arranging the session on nutritional factors in cancer. Dr. Granville Bennett, professor of pathology of the University of Illinois, and Dr. L. Henry Garland, professor of radiology at Stanford University Medical School, jointly will conduct the conference on tumors and other diseases of bone.

The third Bertner Foundation Lecture and Award will be made at the banquet on April 25. All meetings will be held at the Shamrock. Further information may be obtained from William O. Russell, M.D., at 2310 Baldwin Street, Houston 6, Texas.

CORRECTION

In the article entitled "Rachitic Changes in Vertebral Bodies" by Dr. Robert C. Hendrix, published in the February issue, page 174, the footnote "From the Department of Pathology, University of Michigan" was inadvertently omitted.

Books

AUTOPSY DIAGNOSIS AND TECHNIC. By Otto Saphir, M.D., pathologist, Michael Reese Hospital; clinical professor of pathology, University of Illinois College of Medicine, Chicago. Third edition. Pp. 471, with 80 illustrations and 20 tables. Price $6. Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 49 E. 33d St., New York 16, 1951.

The usefulness of this well-known manual has been augmented by another revision and the addition of new material. A modified Virchow technic is described, and the dissection of the heart is illustrated with new figures. Two new chapters of special value have been added: One entitled "'Do' or 'Don't' and Certain Suggestions for Quick Orientation" contains pithy diagnostic hints and aphorisms culled from the author's wide experience; a second, short, chapter, "Suggestions for Evaluation of Autopsies on Embalmed and Decomposed Bodies," offers helpful information not readily obtained elsewhere. The sections on the adrenals and the kidneys have been expanded by comments on pathologic physiology which enliven the descriptive anatomic text. The use of the term "malignant nephrosclerosis" is decried, and "arteriolonecrosis of the kidney" is recommended as designating a histological entity, with full understanding that it represents a complication superimposed on certain other lesions of the kidney. It is questionable, however, that all pathologists will agree with the author in associating arteriolonecrosis most commonly with chronic pyelonephritis.

A few criticisms may be justified. Although many of the older line drawings have been replaced by new ones, the over-all quality of the illustrations is rather poor. Some of the original sketches which have been retained are of dubious teaching value.

With inclusion of an encyclopedic number of diseases, the comments on the gross appearance of organs and the occasional references to etiology and pathogenesis are necessarily brief, and at times telegraphic in style. This results occasionally in generalizations and dogmatic statements that would require elaboration or qualification to avoid their being misinterpreted by the inexperienced reader. There are a few holdovers from older sources that may be questioned, such as the separate descriptions of hypernephroma and carcinoma of the kidney, and the anatomic distinction between fatty degeneration and fatty infiltration of the liver. Some ambiguous and misleading statements are found; for example: The mixed tumor of the parotid is "firm because of the presence of cartilage," and cylindromas of the parotid are "pink endotheliomatous structures"; adenomas and carcinomas of the suprarenal cortex cannot be distinguished on histologic grounds; repeated references to the "submucosa" of the uterus; an increase of fluid in the subarachnoid space is characteristic of cerebral edema; and chondrosarcoma is a very malignant tumor. On page 286, the text should read "mucinous carcinoma of the ovary," instead of "appendix."

These faults do not seriously detract from the value of this volume. In fact, Dr. Saphir's book continues to satisfy a real need as a source of information for students, interns, and residents concerning the performance of the autopsy and the interpretation of the anatomic changes that are observed. It also contains a great deal of information which will aid the pathologist in the performance of his teaching and routine responsibilities.

SURGICAL PATHOLOGY OF THE MOUTH. By E. Wilfred Fish, M.D., L.D.S. (Manch.), D.D.Sc. (Melb.), D.Sc. (Lond.), F.D.S., R.C.S. (Eng.). From the Meyerstein Laboratory for Dental Research, St. Mary's Hospital, Paddington, London. Pp. 463, with 236 illustrations. Price $10. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5, 1951.

The danger and shortcomings of specialization have been recognized for a long time. The present book on the surgical pathology of the mouth represents a sincere attempt to bridge the gap between general biology and pathology and oral biology and pathology. Since it is one of the first in this direction it cannot be expected to do justice to both general and oral pathology. It seems doubtful whether such an undertaking nowadays can be accomplished by any single investigator.

Dr. Fish has achieved in the past an international reputation by his original investigations on the dental pulp and the supporting tissue of the teeth. Both fields of investigation offer unusual opportunities to study the general biology of connective tissue and bone, respectively. Wherever there is an opportunity the author compares changes in the dental and oral tissues with observations of general pathology, as, for example, in the chapters on degeneration, repair, circulatory disturbances, and inflammation. The main part of the book deals with the pathology of the skeleton of the face, the tissues of the tooth, the tooth-supporting apparatus, and the oral mucous membrane. The final chapters discuss the oral tumors.

In evaluating the merits of a book, one has to keep in mind the author's aim in writing it. The author states that the book "arose from an attempt to escape empiricism in everyday dental practice, and it is an account of what appeared to happen when dental or oral tissues were interfered with experimentally, supplemented by deductions arrived at from examination of them when they became diseased." The original viewpoints of the author will certainly stimulate oral surgeons and pathologists to further investigations in this field.

Author and publishers are to be congratulated on the excellent print and reproduction of the illustrations.

PATTERNS OF DISEASE ON A BASIS OF PHYSIOLOGIC PATHOLOGY. By Frank L. Apperly, M.A.M.D. (Oxford), D.Sc. (Melbourne), F.R.C.P. (London), professor of pathology, Medical College of Virginia, Richmond, Va. Pp. 456, with 50 illustrations and 37 charts. Price $8. J. B. Lippincott Company, 227-231 S. Sixth St., Philadelphia 5; Aldine House, 10-13 Bedford St., London, W.C. 2; 2083 Guy St., Montreal, 1951.

The purpose of this book is to present the discipline of pathology from a physiologic point of view. It is especially written for the medical student and the young physician. It deliberately avoids the presentation of gross and microscopic material where such is considered to be of no functional import. It deals with both general and systemic pathology, along the usual divisional and categorical lines of standard texts.

The attempt at a physiologic approach is indeed laudatory, but seems to cover material which is better presented in a physiologic text. On the other hand, the lack of a comprehensive gross and microscopic morphologic discussion makes difficult the presentation of this text to the medical student. There are no gross photographs and no photomicrographs of actual specimens and slides. The diagrams and charts, however, are excellent and instructive.

The text is very well and clearly written, with an obvious pedagogic approach. However, in the opinion of this reviewer, the approach is on an insufficiently high level for the medical student. It can, however, be used profitably in conjunction with a standard morphology-inclined text.

CLINICAL HEART DISEASE. By Samuel A. Levine, M.D., clinical professor of medicine, Harvard Medical School; physician, the Peter Bent Brigham Hospital, Boston; consultant cardiologist, Newton-Wellesley Hospital; physician, New England Baptist Hospital. Fourth edition. Pp. 556, with 192 illustrations. Price $7.75. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

In this fourth edition the author has taken cognizance of the many advances in knowledge of heart disease in the last decade. These concern antibiotic and surgical treatment, as well as the newer knowledge of electrocardiography. The form, approach, and style, however, remain the same. This remains fundamentally a clinical book. References to pathologic phenomena are incidental. The pathologist, however, can find here an excellent source of clinical information of heart disease.

CANCER CYTOLOGY OF THE UTERUS. By J. Ernest Ayre, M.D., director, Dade County Cancer Institute, Cancer Cytology Center, and chief, Cancer Research Department, Medical Research Foundation of Dade County, Miami, Fla. Pp. 407, with 362 illustrations. Price $14.50. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

This beautifully compiled book may rightly be called "A Picture Book of Uterine Cytology." Almost all of the 362 pictures are clear and readily diagnosed. Only a few of the histologic

reproductions do not meet this standard. This is particularly regrettable, since some of these are used to show how difficult it sometimes is on histologic grounds to differentiate between intra-epithelial carcinoma, basal cell hyperplasia, etc. If the reviewer received a slide which was the equivalent of such a picture, he would refuse to diagnose it and insist on a better preparation. There is little actual text. The pictures are not only described in great detail, but, when deemed necessary, the descriptions are accompanied by short histories and follow-ups. Very often the term "nearo-carcinoma" is used and defined as incompletely developed cancer cells, i. e., the group of cells that has been described as the "precancer cell complex." It is questionable whether the current vocabulary which already contains such dubious terms as "precancer," "pre-invasive cancer," and "carcinoma in situ" should be further enriched. Besides, almost everybody would pronounce it as rhyming with "zero" and create the false impression of a condition "near to cancer," while it probably should be pronounced "nearo" (meaning earliest stage). Excellent and to the point is the chapter on definitions of common cytologic terms. It does not seem quite right for the author to cite as proof of a certain point the work of others who quote him as the source of their information.

As the volume stands it probably will be profitably read by gynecologists who would like to do their own cytologic examinations and by technicians who might have to do cytologic examinations. A pathologist would be more interested in a book which discusses vital lesions such as "carcinoma in situ" and precancerous lesions much more critically, and which is written from the point of view of a pathologist also familiar with similar lesions in other regions of the body.

AN INTRODUCTION TO UNIVERSAL SEROLOGIC REACTION IN HEALTH AND DISEASE. By Reuben L. Kahn, D.Sc., University of Michigan Medical School and Hospital, Ann Arbor, Mich. Pp. 158, with 113 illustrations. Price $3.50. The Commonwealth Fund, Division of Publications, 41 E. 57th St., New York 22, 1951.

The author's thesis is that certain lipids liberated from tissue stimulate the production of antibodies even in normal persons. In the presence of certain diseases, notably syphilis, leprosy, and tuberculosis, the liberation of lipids is increased and there is a corresponding increase in the production of antibodies. The presence of these antilipid antibodies may be demonstrated by setting up multiple tests in which different serum dilutions and salt concentrations are employed. The resulting curves are usually similar if the serums are from normal individuals, but those of a few persons show an unusually great reactivity. Patterns of reaction curves differ in the several diseases studied by the author or in the different stages or types of a single disease.

It is suggested by the author that the universal serologic reaction may prove to be a diagnostic tool for studying and differentiating many diseases. That it might be used in studying heredity, aging, and other factors in normal populations is also suggested.

The author attributes the universal serologic reaction to multiple reactions between the many lipid antigens in heart extract antigen (Kahn antigen, for example) and antibodies against similarly diverse lipid antigens liberated by tissue. If this hypothesis holds, it would seem desirable to carry on further studies with a variety of separate lipid antigens rather than with a gross mixture such as exists in Kahn antigen.

PERSPECTIVES IN HUMAN MALNUTRITION: A CONTRIBUTION TO THE BIOLOGY OF DISEASE FROM A CLINICAL AND PATHOLOGICAL STUDY OF CHRONIC MALNUTRITION AND PELLAGRA IN THE AFRICAN. By Joseph Gillman, D.Sc., M.B., B.Ch., and Theodore Gillman, M.Sc., M.B., B.Ch., Departments of Physiology and Anatomy, Medical School, University of the Witwatersrand, Joint Nutrition Unit of the Council of Scientific and Industrial Research, and the University of Witwatersrand, Johannesburg, South Africa. Pp. 584, with 259 illustrations, 45 tables, and 3 charts. Price $18. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

The late George R. Minot used to tell his patients that the place to buy vitamins was the grocery store—not the drugstore. Arnold P. Meiklejohn, his pupil, was one of the first to point out that malnutrition could produce reversible biochemical lesions in the body as well as irreversible tissue changes in specific organs. Meiklejohn coined the useful term "metapathy"

to describe the changeable biochemical aberrations of deficiency. He also showed that the irreversible specific cellular damage of malnutrition could induce, in man, a state of generalized nutritional bankruptcy which ultimately implicated organs far removed from those originally harmed by dietary restriction. The concept that "good" food is better for man than vitamins and the idea that malnutrition can harm the whole body is the central theme of the book "Perspectives in Human Malnutrition" by Drs. J. and T. Gillman. Minot and Meiklejohn presented the concepts described above in succinct papers. The Gillmans' book is prolix and filled with unsubstantiated speculations. This detracts immeasurably from the magnificent pictures of malnourished Africans and the fine reproductions of pathologic changes in their tissues and organs.

The lesions described in this book, such as hemosiderosis and infantile pellagra, will be of interest to pathologists and clinicians who are not versed in tropical or exotic diseases. The reports of a poor response in the African pellagrin to therapy with nicotinic acid will come as a surprise to nutritionists in this country, especially in the light of Goldsmith's recent experimental production and cure of full-blown pellagra in man by dietary manipulations of nicotinic acid.

The Gillmans put forward many other unorthodox ideas and have labored from the literature to press home their points. For example, they propose the use of Speransky's spinal pumping as a therapeutic measure in rheumatoid arthritis. The patient is "cured" by pumping his cerebrospinal fluid in and out of his spinal canal. The hypothetical background for this procedure was Speransky's "Theory of Medicine," which is closely akin to the theories of Palmer, the founder of chiropractic.

Hypotheses and unorthodox ideas are beacons which may light the way to truth, but real advances in science come from careful observation and properly controlled investigations. The histologic observations reported in this book warrant careful consideration, but much of the data the Drs. Gillman present here and elsewhere on therapeutic investigations in man are drawn from investigations poorly planned and uncontrolled. Even their nutritional studies in animals lack the usual inanition controls.

The African south of the equator suffers and dies from tuberculosis, syphilis, acute infectious disease, and parasitic infestations, as well as from malnutrition. It would have been extremely valuable if the Drs. Gillman had been less preoccupied with speculation and had presented detailed protocols of the autopsies made on their patients who died with pellagra and other nutrition syndromes, since these protocols do not appear in their publications. If this would have taken up too much room, tables of primary and secondary causes of death would have given worthwhile data on the interrelations between dietary disease and other causes of ill-health.

In the final chapter of their book they make a well-intentioned, humanitarian, but impractical plea for an even distribution of food between the white and black races in South Africa. The report of Brock and Autrets to the World Health Organization last year on malignant malnutrition in Africa demonstrates clearly the urgent need for attacking malnutrition in Africa by every possible means, including fortification of basic cereal diets with synthetic vitamins and natural protein-rich food supplements. This the Gillmans condemn on the basis of inadequate studies in the rat, an animal whose requirements for nutrients, such as methionine, are quite different from those of man.

A Textbook of Clinical Neurology. By Israel S. Wechsler, M.D., clinical professor of neurology, Columbia University, New York; consulting neurologist, The Mount Sinai Hospital, Montefiore Hospital, and Rockland State Hospital, New York. New seventh edition. Pp. 801, with 179 illustrations. Price $9.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1952.

Infective Hepatitis: Studies in East Anglia During the Period 1943-47. By F. O. MacCallum, A. M. McFarlan, J. A. R. Miles, M. R. Pollock, and C. Wilson. Medical Research Council Special Report Series, No. 273. Paper bound. Pp. 144, with 21 illustrations and 61 tables. Price 4 shillings 6 pence net. His Majesty's Stationery Office, York House, Kingsway, London, W.C. 2, 1951.

THE BATTLE FOR MENTAL HEALTH. By James Clark Moloney, M.D. Pp. 105. Price $3.50. Philosophical Library, Inc., 15 E. 40th St., New York 16, 1952.

BULLETIN OF THE HOSPITAL FOR JOINT DISEASES—HENRY L. JAFFE ANNIVERSARY VOLUME. Thirty-five contributors. Vol. 12, No. 2. Pp. 527, with numerous illustrations. Price $6. Williams & Wilkins Company (Waverly Press), Mount Royal & Guilford Aves., Baltimore 2, October, 1951.

DIE CHIRURGIE DER SPEICHELDRÜSEN. By Prof. Dr. E. König. Pp. 123, with 44 illustrations. Price 12 German marks. Walter De Gruyter & Company, Genthinerstrasse 13, Berlin W 35, 1951.

LIVER DISEASE. A CIBA FOUNDATION SYMPOSIUM. Consulting editor, Sheila Sherlock, M.D., F.R.C.P. Editor for the Ciba Foundation, G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch. Pp. 249, with 112 illustrations and 35 tables. Price $5. The Blakiston Company, Inc., 1012 Walnut St., Philadelphia 5, 1951.

LEHRBUCH DER RÖNTGENDIAGNOSTIK. By H. R. Schinz, W. E. Baensch, E. Friedl, E. Uehlinger. Paper bound. Pp. 463, with 522 illustrations. Price 84 German marks. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart O; agents for U. S. A., Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

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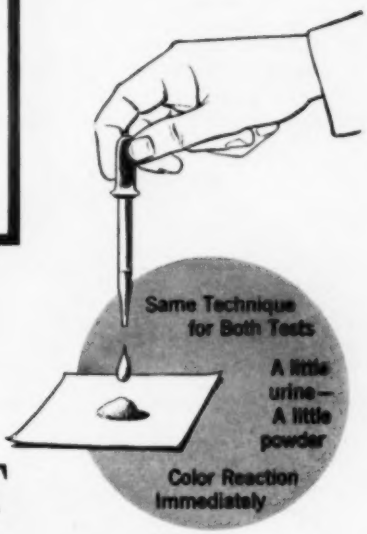

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"Cortisone"—J. H. Carlisle, M.D., A. Gibson, M.D., E. Schmaeilla, M.D. —*Postgraduate Medicine, Aug., 1950.*

"Oral Cortisone Therapy in Intractable Bronchial Asthma" — E. Schwartz, M.D. — *Journal of the American Medical Association, December 29, 1951.*

"Cortisone (Compound E), Summary of its Clinical Uses"—J. H. Carlisle, M.D.—*British Medical Journal, September 9, 1950.*

"Cortisone and ACTH—A Review of Certain Physiologic Effects and Their Clinical Implications" — Randall G. Sprague, M.D.—*American Journal of Medicine, May, 1951.*

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*Bronstein, M. R., and Witkind, E.: Am. J. Med. Sci. *222*, 677, Dec., 1951.

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